Examining the feeding ecology of two mesopelagic fishes (*Lampanyctodes hectoris* & *Maurolicus walvisensis*) off the west coast of South Africa using stable isotope and stomach content analyses

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Declaration

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Abstract

Although mesopelagic fishes are an important component of marine food webs, the adaptive features used to facilitate niche partitioning among co-existing and presumably competing mesopelagic species is unclear. This study examined the trophic ecology of the two principal mesopelagic fishes off the west coast of South Africa (lanternfish *Lampanyctodes hectoris* and lightfish *Maurolicus walvisensis*) sampled during the spring 2014 and autumn 2015 cruises, using stable isotope and stomach content analyses. Stable isotope values were extracted from the white muscle tissue of fishes, but due to the high lipid content of both species, samples were processed in duplicate: $\delta^{13}C$ was measured from lipid-extracted samples and $\delta^{15}N$ from non-extracted samples. To validate the stable isotope results, stomach contents were examined and the relative importance of prey items was assessed using three measures: frequency occurrence (%F), numerical abundance (%N), and dietary carbon (%C).

Both mesopelagic species occupied different isotopic niches that were separated by their $\delta^{15}N$ values across a similar $\delta^{13}C$ range. Furthermore, the relationship found between trophic position and standard length emphasizes the structuring effect of size within the assemblage, with the larger species (*L. hectoris*) occupying a higher trophic position than the smaller species (*M. walvisensis*). Although copepods dominated the diet of *L. hectoris* in terms of numerical abundance (42%), macrozooplankton was by far the most important dietary component, with euphausiids contributing 53% of dietary carbon. Conversely, copepods – particularly *Calanus* sp. – were the most important component of the diet for *M. walvisensis* in terms of their occurrence (84%), numerical abundance (64%), and dietary carbon (67%).

Though some dietary overlap exists between *L. hectoris* and *M. walvisensis*, the results of this study suggest resource partitioning within the mesopelagic assemblage, likely facilitated by differences in alimentary morphology (i.e. trophodynamically mediated), and possibly by differences in their respective foraging strategies. Similarly, ontogenetic shifts in trophic position were detected, which suggests that these adaptive features may also be used to mitigate intra-specific competition within populations. Furthermore, the trophic positions of both *L. hectoris* and *M. walvisensis* inferred from dietary and isotopic data signify that mesopelagic fishes (in the context of this study) are secondary and tertiary consumers in the marine ecosystem of the southern Benguela. Nevertheless, samples covering a larger area of the southern Benguela and multiple years would be needed for a more complete understanding of the trophic ecology of these two species.

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Chapter 1: Introduction

1.1 Study context

Mesopelagic fishes constitute an important component of pelagic ecosystems due to their high biomass [1], vertical diel migrations [2], and global distribution [3]. In South Africa, the mesopelagic assemblage is dominated by lanternfish *Lampanyctodes hectoris* and lightfish *Maurolicus walvisensis,* which form dense aggregations over the upper continental slope in the northern and southern Benguela sub-systems [1,4]. Although they are consumed by large demersal and pelagic fishes [5,6], cephalopods, marine birds [7], and marine mammals [8], they may also exert notable feeding pressure on lower trophic levels due to their high abundance [9]. Yet, despite playing an important role in marine trophodynamics, little is known about their basic biology or feeding ecology in the Benguela Current [1,10].

Trophic relationships are fundamental to the understanding of biological interactions within an ecosystem. Consequently, the purpose of this study was to examine the feeding ecology of *L. hectoris* and *M. walvisensis* sampled off the west coast of South Africa through the use of stable isotope and stomach content analyses. To date, no isotopic investigations for mesopelagic fishes around South Africa are available in the scientific literature. Therefore, the first aim of this study was to define (and compare) the isotope niches and trophic positions of *L. hectoris* and *M. walvisensis* through stable isotope analysis. Though the diet of *L. hectoris* was described to some extent for populations off South Africa [11,12] and south east Australia [13] between 1986 and 1987, updated dietary information for *L. hectoris* is presently unavailable in the literature. Similarly, the diet of *M. walvisensis* has yet to be described, though the feeding ecology of a closely related congener *M. muelleri* has been well studied elsewhere [14-17]. As a result, the second aim of this study was to quantitatively describe their diet compositions and assess the relative dietary importance of different prey in terms of their frequency of occurrence, numerical abundance and carbon content. Thirdly, this study examined the trophodynamics of mesopelagic fishes derived from dietary and isotopic data in the context of the west coast sub-system of the southern Benguela

1.2 A review of the mesopelagic fishes in the southern Benguela

1.2.1 The Benguela Current & ecosystems

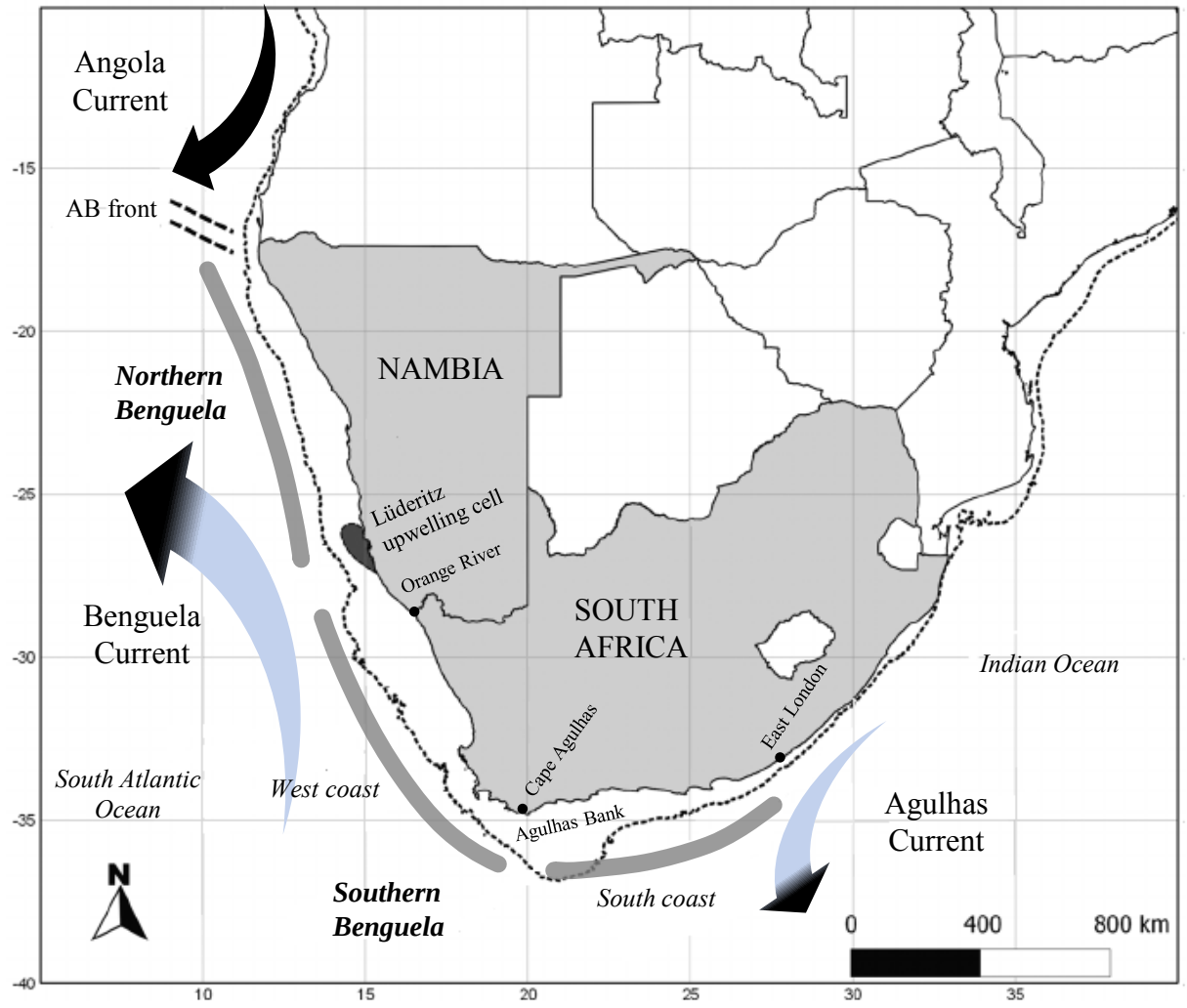


Fig.1.1. Map of the Benguela Current bordering Namibia and South Africa, showing the 500-m depth contour (dashed line) and the locations of the Angola Current, the Angola-Benguela (AB) Front, the Lüderitz upwelling cell (which separates the northern and southern Benguela systems), and the Agulhas Current. The west coast and south coast subsystems of the southern Benguela are shown. Image modified from Roux *et al*. [19].

Located off the southwestern coast of Africa (Fig.1.1), the Benguela Current ecosystem is one of the four major eastern boundary upwelling systems of the world [18]. As is typical of these systems, it is characterized by wind-driven pulse upwelling and resultant high productivity [18,19]. The Benguela Current itself extends from the Cape Peninsula in the south to the Angola-Benguela front in the north (usually located between 14°S and 16°S) [18]. The Benguela ecosystem is divided into two systems, the Northern Benguela (southern Angola and Namibia) and the

Southern Benguela (South Africa), separated by the permanent upwelling cell off Lüderitz (Fig. 1.1) [18,20]. The strong winds, turbulent mixing, and offshore transport associated with the Lüderitz upwelling cell is thought to act as a barrier between some northern and southern fish stocks [18]. As a result of the upwelling cell, the two systems differ significantly in both their physico-chemical and biological characteristics [18].

Encompassing the upwelling region from the Lüderitz upwelling cell southward, the southern Benguela also extends over the Agulhas Bank to East London (28°E) [18]. The southern Benguela is further divided into two sub-systems (Fig. 1.1). The first is the coastal upwelling system off the west coast of South Africa, which is characterized by seasonal, wind-driven upwelling at discrete points and high productivity [18,19]. The second is off the south coast and constitutes a temperate shallow system over the Agulhas Bank that displays coastal, shelf-edge and dynamic upwelling, and moderate productivity [18,21,22].

1.2.2 The mesopelagic Myctophidae & Sternoptychidae

Mesopelagic fish are generally defined as species which predominantly inhabit the mesopelagic 'twilight' zone and situated seaward of the continental shelf break [4]. With a global biomass estimated at roughly 1,000 million metric tonnes [3,23], mesopelagic fish are found throughout the world's oceans from the Artic to the Antarctic; however, species abundance and production is greatest in tropical and subtropical regions [3]. Over 700 species are found within the mesopelagic zone, and many teleost families fall under this definition, but the greatest diversity is represented by the Myctophidae (lanternfish), Gonostomatidae (bristlemouths), Sternoptychidae (hatchetfish and lightfish), and Paralepididae (barracudinas) [3,4].

The lanternfishes (myctophids) are the most widespread and speciose (approximately 250 species in 30 genera) of the mesopelagic fishes [24], with a global biomass estimated at 550 to 600 million metric tonnes [3]. As such they not only play an important role in energy cycling throughout marine food-webs, but also represent a potential alternate resource for commercial exploitation [3]. In comparison, lightfishes (sternoptychids) consist of 73 species in 10 genera and are less abundant relative to myctophid species [3]. Although the estimated global biomass of lightfishes is presently unavailable in the literature, Hulley & Prosch [4] estimate that the biomass of the oceanic mesopelagic assemblage in the South Atlantic comprises of 50 to 60 per-

cent myctophids and only 4.7 percent sternoptychids. Compared to ubiquitous myctophids, the distribution of sternoptychids is also relatively limited, with species aggregating near continental slopes in tropical, subtropical and temperate regions of the Atlantic, Pacific, and Indian Oceans [15].

1.2.3 Lanternfish & lightfish of the southern Benguela

The myctophid, *Lampanyctodes hectoris* is associated with land masses near the subtropical convergence and is widely distributed off Chile, New Zealand, Australia and South Africa [11,25]. By contrast, the sternoptychid *Maurolicus walvisensis* is limited in its distribution and is found solely along the continental shelf of southern Africa and seamounts in the south west Indian Ocean [26]. Maps of the global distribution for *L. hectoris* and *M. walvisensis* are provided in Appendix A. Of the 65 myctophid species (25 genera) recorded in the southern Benguela, *L. hectoris* is the most abundant species [27]. By contrast, *M. walvisensis* is the only sternoptychid species recorded in the region[1] [4]. Coetzee *et al*. [1] estimated that the total biomass of mesopelagic fish in the west coast sub-system of the southern Benguela to be in the order of 1.2 million tonnes (as of Spring 2006), of which *L. hectoris* and *M. walvisensis* (and to a lesser extent *Symbolophorus boops*) were the most abundant species. Due in part to their high abundance in the region (as well as their high lipid content), mesopelagic stocks have been experimentally and commercially exploited in the southern Benguela since the early 1960s, a summary of which is provided in Appendix B.

Though the distribution of mesopelagic fishes in southern Benguela exhibits spatio-temporal variation due to their respective migratory behaviour [2,4], they nevertheless tend to concentrate in areas where strong upwelling occurs, i.e. around the Cape Point, Cape Columbine, Hondeklip Bay, and Lüderitz upwelling cells (Fig. 1.2) [4]. Earlier efforts to map the distribution of mesopelagic fishes in the southern Benguela used commercial catch data of *L. hectoris* from the early 1970s [28]. Highest catches were recorded from Cape Point to St. Helena Bay and observations via spotter plane (hired by the fishing industry) confirmed this distribution over a

[1] Within the family Sternoptychidae, the genus *Maurolicus* was conventionally considered to be monotypic and represented by a single species, *M. muelleri*. In the mid 1990's, Parin & Kobyliansky [15] revised the genus and recognized fifteen allopatric species based on their meristic and morphological characteristics, as well as their distribution patterns. Mesopelagic studies in the Benguela Current prior to the genus revision reported the stermoptychid *M. walvisensis* as *M. muelleri*.

number of years (1974-1983) [28]. Recent efforts to update stock biomass estimates and distributions of mesopelagic fishes from Walvis Bay to Cape Point were undertaken in spring 2006 using an acoustic and trawl survey [1], which provided distribution patterns similar to historical records. In the west coast sub-system of the southern Benguela, *L. hectoris* densities were lower than those recorded in the northern Benguela and limited to the offshore regions between Cape Point and Doring Bay (Fig. 1.2a), with high-density aggregations between Cape Point and Cape Columbine [1]. The distribution of *M. walvisensis*, by contrast, expanded across the shelf in most areas of the west coast, and increased towards the south where several high-density aggregations were recorded in the mid-shelf area south of Hondeklip Bay (Fig. 1.2b)[1].

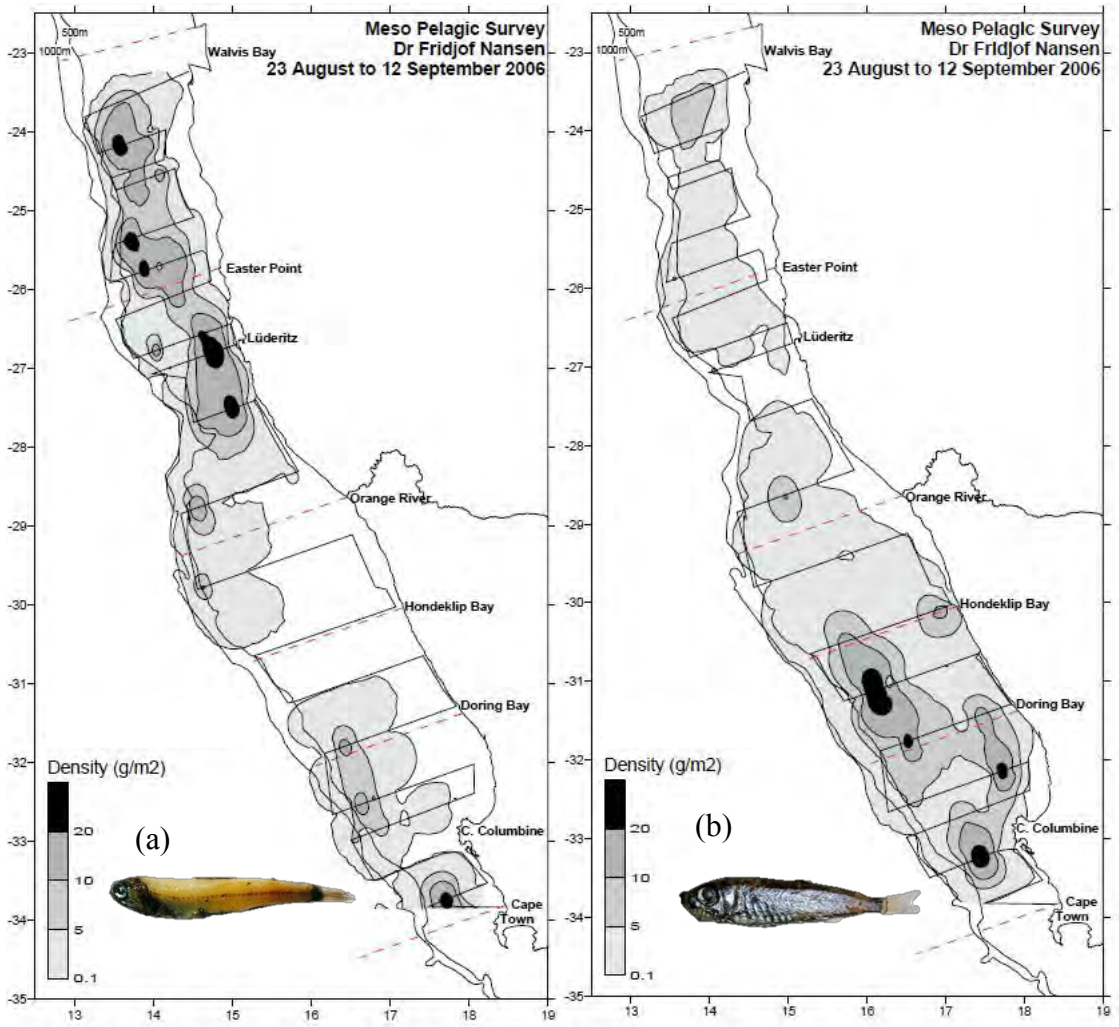


Fig. 1.2. Distribution and relative abundance of (a) *Lampanyctodes hectoris* and (b) *Maurolicus walvisensis* acoustically surveyed from August through September (spring) 2006, figures from Coetzee *et al* [1].

Except for features directly linked to classification, anatomical and physiological studies on mesopelagic fish in the southern Benguela, and elsewhere, are few. The lanternfish *L. hectoris*, so named for the light-organs on the head and body, is a small, slenderly compressed fish, with a maximum standard length (SL) of 73 mm (Fig. 1.3a) [24]. It has a prominent, bluntly rounded head, with large round eyes and a terminal mouth (Fig. 1.3a). By contrast, *M. walvisensis* is the smaller of the two species (max. 47 mm SL) and is laterally compressed with a pointed snout, small eyes, and a superior (surface oriented) mouth (Fig. 1.3b) [15]. Like most mesopelagic fishes, both species are R-selected: they are short-lived (1-5 years) and characterized by rapid growth, early sexual maturation, and high mortality rates [11,24,29]. A full comparison of meristic, morphological, and life history traits of the two species is provided in Appendix A.

Fig. 1.3. The appearance of (a) *Lampanyctodes hectoris* (64.5mm SL) and (b) *Maurolicus walvisensis* (41.6mm SL) sampled from the west coast of South Africa, shown to scale.

1.2.4 Trophic ecology of mesopelagic fishes

Although mesopelagic fishes constitute a major part of the biomass in the southern Benguela [20], their position in the food web is poorly understood [10]. Lanternfishes and lightfishes are generally classified as opportunistic planktivores that feed predominantly on crustaceans (copepods, euphausiids, ostracods, amphipods) and to a lesser extent on chaetognaths, fish larvae and fish eggs [3,11]. Studies indicate that mesopelagic fish are important predators of zooplankton [6] and in some areas possibly exert strong top-down control on lower trophic levels [9]. However, mixed mesopelagic assemblages (i.e. consisting of fish varying in age, size, species, geographic and/or genetic origins) are not uncommon and how they manage to coexist is an interesting question, and one this study seeks to elucidate.

Resource partitioning refers to an evolutionary change in the resources used by species in response to selection pressures generated by interspecific and intraspecific competition [30]. While Tyler & Perry [31] found no indication of resource partitioning between mesopelagic fish-

es off the coast of Oregon, similar studies in Hawaii [32], Gulf of Mexico [33,34], Tasmania [35], and the Southern Ocean [7] documented some degree of dietary segregation among mesopelagic assemblages. These studies largely attributed the observed dietary differences to the morphological characteristics, feeding strategies, and/or geographical (vertical and horizontal) distributions of the species examined. For instance, Hopkins & Gartner [34] attributed niche segregation between several myctophid species in the eastern Gulf of Mexico to differences in habitat and food availability. Clarke [32] however suggested that the observed dietary differences between 16 species of mesopelagic fishes from Hawaiian waters were regulated by two morphological features, namely the size of the eye lens and gill-raker spacing. By contrast, resource partitioning among mesopelagic fishes in the southern Benguela has yet to be investigated. Rather, much of the literature from the region has focused on the mechanisms which mediate resource partitioning among commercially important small pelagic forage fishes [36-39].

Mesopelagic fishes are known to undertake extensive vertical diel migrations, ascending at night as they follow zooplankton on which they feed [3]. As a result, these small forage fish play a critical role in the trophodynamics of marine food webs. They contribute to nutritional and energetic exchanges between lower and higher trophic levels, as well as between shelf and deep-sea ecosystems [3,6]. In the southern Benguela, predation mortality for *L. hectoris* and *M. walvisensis* is purported to be high at the shelf edge where the distributions of neritic piscivores and mesopelagic fishes overlap [11]. Both species are preyed on by other mesopelagic fishes like dragonfishes (the Stomiidae family) [40], by larger pelagic fish that forage off the shelf (snoek, tuna, swordfish, mackerel, etc.) [6,11], and by demersal fishes that undertake vertical migrations to feed on pelagic prey (deep- and shallow-water hakes, Cape dory, etc.) [5,6]. Mesopelagic fishes also represent a potentially important resource used by sea mammals (e.g. pinnipeds and cetaceans) [41] and are thought to be of potential importance to seabirds around southern Africa (e.g. petrels and shearwaters) [11,42].

In an effort to understand why and how predators select their prey, a general theory of optimal foraging (OFT) was developed. The premise of OFT is that natural selection will favour predators whose feeding strategies maximize their net energy intake per unit time of foraging [43, 44]. The proximate lipid concentration and relative caloric value of *L. hectoris* and *M. walvisensis* were found to be significantly higher than other forage fishes (clupeids and engraulids)

that otherwise dominate the region in terms of their abundance [11]. To an extent, the literature supports OFT and suggests that mesopelagic fish can play an important role in the food web of the Benguela, particularly as a link between zooplankton and hake [10,20]. For instance, off the west coast of South Africa *L. hectoris* and *M. walvisensis* were the dominant component (78%) of the diet for small deep-water hake and remained important for hake up to 60cm in length [5]. Consequently, mesopelagic fishes likely represent a significant, if alternate, energy source for predators in the Southern Benguela, and possibly exert strong bottom-up control over commercially important fish [36]. Both anchovy and sardine stocks have shown variability in their abundance and distribution off South Africa in recent decades (Fig. 1.4) [39,45]. As a result, mesopelagic fishes likely represent an important prey resource off the west coast when the availability of pelagic forage fish is otherwise limited [4,46]. These lesser known alternative pathways to the traditionally studied small pelagic-predator links are probably critically important in maintaining ecosystem structure. Therefore, population variability of these few but abundant mesopelagic and pelagic forage fishes, whether through environmental or anthropogenic forcing, could influence the ecosystem as a whole [9,24].

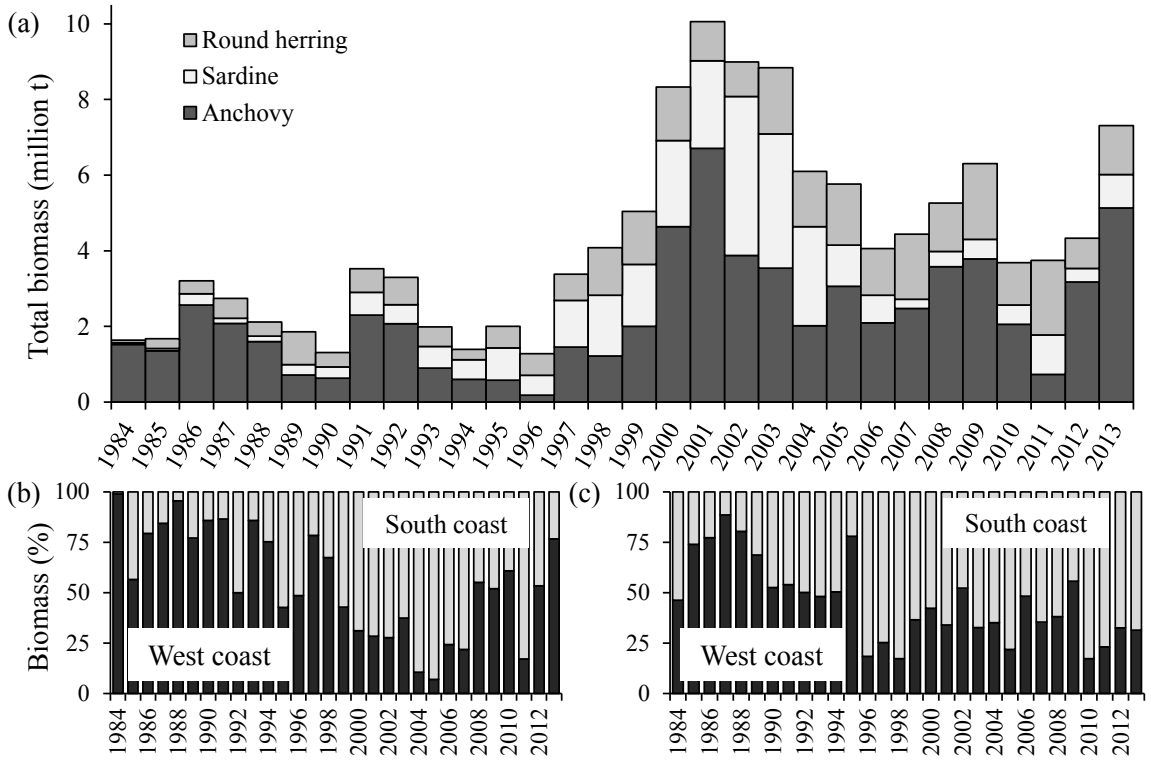


Fig. 1.4. Acoustically-estimated (a) total biomass of anchovy *Engraulis encrasicolus*, sardine *Sardinops sagax* and round herring *Etrumeus whiteheadi* from 1984 to 2013, as well as the percentage of the total biomass located on the west and south coasts of the southern Benguela for (b) sardine and (c) anchovy during this time period. Data was extracted from the DAFF 2014 Status Report [45].

1.3 Methodological approaches used to investigate trophic ecology

1.3.1 Trophic ecology

Trophic levels define the position of organisms within a food web. Estimates of trophic position can be used to calibrate and validate food web models, to determine the extent of trophic interactions (i.e. predator-prey interactions), and to calculate energy transfer efficiency from one level to the next [47, 48]. They can also be used as indicators of food web structure, ecosystem function, and anthropogenic impacts [10, 20, 49] and therefore contribute to the knowledge base for the implementation of an ecosystem approach to fisheries (EAF) management. The trophic ecology of organisms can be assessed in one of two ways: directly, through stomach content analysis and stable isotope analysis, or indirectly, through food web models that use diet or stable isotope data as inputs [49]. Consequently, the scope of this review is on the use of both stable isotope and stomach content analyses in a trophoecological context.

1.3.2 Stable isotope analysis

Stable isotope analysis has become an important tool with a great number of applications [50]; however the focus of this review is on its application in trophic ecology. Of the commonly occurring stable isotopes, those of carbon and nitrogen are most frequently used to this end. By examining the ratio of heavy to light isotopes for carbon and nitrogen it is possible to reconstruct diets, estimate trophic positions, elucidate energy flow through food webs, and assess ontogenetic and niche shifts amongst others [50-53]. These applications take advantage of the natural variations in stable isotope abundances, where organisms preferentially sequester heavy isotopes, i.e. ^{13}C and ^{15}N, relative to light isotopes, i.e. ^{12}C and ^{14}N, with each trophic transfer through isotopic fractionation [54, 55]. There are several possible biological processes that could contribute to this enrichment: (1) preferential loss of light isotopes during respiration, (2) preferential sequestration of heavy isotopes during digestion and/or assimilation, and (3) metabolic fractionation between different tissue types [55-57].

Carbon stable isotope signals ($\delta^{13}C$ or $^{13}C/^{12}C$) undergo minimal trophic fractionation (0.5-1.1‰ increase between diet and consumer)[58] and help estimate the source production (i.e. origin of organic matter) in a given food web. Since primary producers have distinct $\delta^{13}C$ signals due to their photosynthetic pathway, this gives rise to the observed differences in ^{13}C /^{12}C

throughout a food web [58,59]. Unlike carbon, which has multiple macromolecular dietary sources (carbohydrates, lipids, etc.), nitrogen in the consumer's muscle tissue is almost entirely supplied by dietary protein in the form of amino acids [51]. Nitrogen signals ($\delta^{15}N$ or $^{15}N/^{14}N$) tend to increase with each trophic transfer in a predictable manner (+3.4‰) [57]; however, a wide range of fractionation rates for nitrogen have been reported in marine systems. Although nitrogen signals provide a robust measure of an organism's trophic level within the food web [51], one must consider that a consumer's $\delta^{15}N$ reflects the composition of an assimilated diet, integrated over time [49]. To estimate a consumer's trophic level, $\delta^{15}N$ signals must be compared against a $\delta^{15}N$ baseline, which is difficult to assess because different supporting food webs may themselves differ in their $\delta^{15}N$ signals [49].

In systems where few sources of production dominate (i.e. pelagic systems), the main uncertainties stem from seasonal and spatial variation in the $\delta^{15}N$ baseline [49]. Though phytoplankton production is the ultimate source of production in the open ocean, the $\delta^{15}N$ of phytoplankton and their small zooplankton consumers are highly variable, limiting their use as reliable baseline indicators [49]. Because these organisms are short-lived and exhibit rapid turnover rates, they may not be suitable baseline indicators for long-lived secondary consumers like fish, which assimilate dietary carbon and nitrogen over longer time scales [49]. Thus, the $\delta^{15}N$ of sedentary filter-feeding bivalves (with slow turn over times) are increasingly being used to establish ecosystem baselines [49,60,61], following the recommendation of Cabana & Rasmussen [62]. These organisms are thought to integrate the high frequency and small-scale variation in the isotopic signatures of their phytoplankton and zooplankton diets [49]. In the Benguela ecosystem, isotopic studies of organisms at or near the base of the food web are limited. Two studies are available from the northern Benguela system; the first measured the $\delta^{15}N$ of surface sediment organic matter [63] and the second measured the $\delta^{15}N$ of mixed phytoplankton near Walvis Bay [64]. In the southern Benguela, only two sources are available in the literature, both of which measured the $\delta^{15}N$ of the littoral mussel *Mytilus galloprovincialis* off the west and south coasts of South Africa [65,66].

1.3.2.1 Lipid correction methods: lipid extraction vs. mathematical models

Mesopelagic fishes possess high lipid concentrations for which they are exploited at a commercial scale (Appendices A and B) [4]. However, these lipids are of concern as their presence in tissue samples may bias the $\delta^{13}C$ signals of the muscle tissue. Lipids typically represent a mixture of non-polar (i.e. glycerides) and polar (i.e. fatty acids and wax esters) compounds that can be chemically removed through solvent extraction [52], where compounds are separated based on their relative solubility in two different immiscible solvents [67]. Several procedures have been suggested for extracting lipids from tissue samples prior to stable isotope analysis, including the Soxhlet extraction with chloroform or diethyl ester [68], or exposure to chloroform/methanol mixtures like those used by Folch *et al*. [69] and Bligh & Dyer [70]. Such techniques are advantageous as they remove the majority of lipids from the tissue, producing samples that are otherwise homogenous (i.e. standardized for intra- and inter-specific comparisons) [52]. However, these methods are time-consuming, costly, and can introduce their own artefacts [52]. As these solvents are not lipid-specific, some nitrogenous compounds are removed during lipid extraction, thereby altering the $\delta^{15}N$ values [67]. Although the magnitude of change is often small, it is still greater than the analytical error for $\delta^{15}N$ and best practice suggests analyzing samples in duplicate to derive accurate $\delta^{13}C$ (lipid-extracted) and $\delta^{15}N$ (non-extracted) values for tissues with C:N ratios great than 3.5 [52, 67].

To avoid chemical correction, several mathematical models have been developed. Mathematical normalization provides some advantages over chemical lipid extraction, as this simplifies sample preparation, reduces analytical costs, and better preserves the integrity of samples for $\delta^{15}N$ analysis [71]. Most mathematical models relate lipid-extracted and non-lipid-extracted $\delta^{13}C$ values to the sample's C:N ratio (or %C) and fall into three major categories: linear, mass-balance, or non-linear models. For more specifics on mathematical correction models see reviews by Fry [51], de Lecea & de Charmoy [52], and Sweeting *et al.* [72]. Such mathematical models are not widely used, however, due to concerns over their generality and lack of rigorous evaluation [52,71]. Additionally, most models were developed for the correction of $\delta^{13}C$ in specific aquatic species; therefore extending their use to different species, taxa, and even tissues, can result in biased or inaccurate $\delta^{13}C$ estimates [52]. Although chemical lipid extraction and mathematical normalization both reduce biases in $\delta^{13}C$, the former is thought to be the most appropri-

ate and reliable method available [52]. Of the delipidation methods examined, Folch *et al*. [69] offer the most exhaustive extraction approach, removing the highest amount of lipid, and is therefore considered the most suitable method available [52, 67].

1.3.3 Stomach content analysis

Diet studies through stomach content analysis have been a standard practice to determine the relative importance of prey and investigate food web dynamics in order to model ecosystem functioning. A number of methods exist to quantify the stomach content of fishes, which range from simple numerical measures to more complex indices of prey importance [73]. The most common methods include: percentage by number (%N), percentage by volume (%V) or weight (%W), and frequency of occurrence (%F) [74].

Each measure provides different insights into the trophic ecology of the species in question; numerical abundance elucidates feeding behaviour, while volume or weight measures reflect the nutritional value of prey [73]. Unlike the other methods, frequency of occurrence does not describe the diet of individuals, but instead represents population-wide food habits [73]. Yet these traditional methods fall short of depicting the true relative value of prey [75] and no method is without bias [73]. For instance, numerical measures over-emphasize small prey when considerable size variation exists, and as a result, this method is most suitable in cases where prey are similarly sized [73]. Although volume and mass avoid bias towards small but numerous prey, these measures of dietary importance are sensitive to large but rare prey items, particularly when samples sizes are small, or may be distorted by differential digestive rates [75]. Similarly, frequency of occurrence is sensitive to sampling error and may over-represent prey that are present only as traces and/or persist longer in stomachs than others [73]. Consequently, a number of compound indices that incorporate two or more of these measurements have been developed to better reflect dietary habits [75]. For instance, food items can be converted to pre-ingestion values if properties (e.g. length to weight regressions) of the prey are known. These pre-ingestion values can then be used to calculate the energetic contribution, i.e. dietary carbon, of each prey type [73]. Although dietary carbon may accurately depict prey importance, care must be taken, since errors in length/mass or length/volume regressions can introduce bias into the results and these errors can appear in the order of 5-10% but may be larger [76].

Nevertheless, these methods provide but a snapshot of dietary importance, and the limitations, although well understood, have stimulated different opinions on their use [77]. The first is the belief that more detail provides more information. Consequently, compound indices (i.e. dietary carbon) are thought to provide a more balanced representation of dietary importance as they combine values from different sources [77]. However, MacDonald & Green [78] argue that these indices may be redundant, adding little new information when compared with any single measure (i.e. %F), while also confounding multiple sources of error and variation. Similarly, Baker *et al*. [79] argue that simpler methods, such as frequency of occurrence, provide robust data that overcomes many of the limitations of more complex approaches. Because considerable disagreement exist between these perspectives, there is no single correct way to study diets and the method chosen must depend on the goals of each study [73].

1.4 Study objectives

This study aims to examine the feeding ecology of *L. hectoris* and *M. walvisensis* sampled during the spring 2014 and autumn 2015 cruises using stable isotope and stomach content analyses, as well as to examine the trophodynamics of mesopelagic fishes in the context of the west coast sub-system of the southern Benguela. More specifically, study objectives are to:

- Compare the stable isotope signals of *L. hectoris* and *M. walvisensis*, in particular $\delta^{15}N$, for trophic segregation within the assemblage.
- Quantify the diet of the two mesopelagic fishes in terms of the frequency of occurrence, numerical abundance, and contribution to dietary carbon derived from stomach contents.
- Determine if seasonal variability is present in the diet and/or isotopic composition of either species and if size differences may be contributing to patterns observed.
- Investigate methodological application of stable isotope analysis used in this study, namely the effect of chemical lipid extraction on isotopic values of mesopelagic fishes and the effect of using literature derived isotopic baselines to standardize $\delta^{15}N$ values for cross-study comparisons.
- Investigate resource partitioning between the two study species, here achieved by comparing the mean prey lengths ingested, overall dietary diversity, and feeding periodicity of each species, in order to infer their respective feeding strategies.
- Investigate size-related shifts in isotopic ratios, mean prey lengths ingested, and trophic positions for both *L. hectoris* and *M. walvisensis.*
- Compare estimates of trophic position obtained in this study in relation to those derived from ecological models and from recent stable isotope analyses in the southern Benguela.

Chapter 2: Methodology

2.1 Sample collection

Samples were collected from mid-water trawls made during two research surveys around the west coast subsystem of the southern Benguela, both of which were conducted by DAFF.[2] Samples obtained from the 2014 Pelagic Spawner Biomass survey were collected during October and November, and those from the 2015 Pelagic Recruitment survey were collected from May through June. Fish stocks were sampled using an Engels 308 midwater trawl, towed at 3.4-4 knots and over a duration ranging from 27 to 38 minutes at various depths. After capture, fishes were sorted on board and the date, time, catch location, depth, grid and station numbers were recorded. For both *Lampanyctodes hectoris* and *Maurolicus walvisensis*, 50 fish per species were arbitrarily sampled by the research crew from each trawl (first order sub-sampling) and blast frozen in bulk for laboratory analysis (Fig. 2.1; Table 2.1).

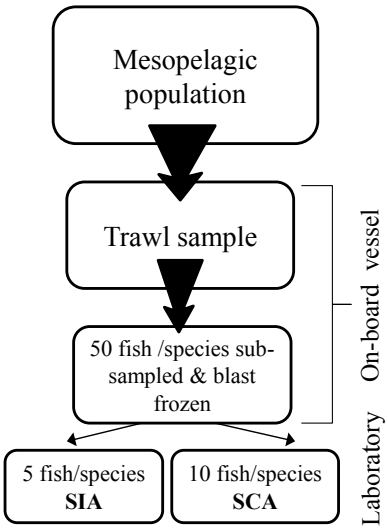


Fig. 2.1. Schematic of the sampling procedure utilized for the purpose of this study.

This study represents a preliminary investigation into the feeding ecology of *L. hectoris* and *M. walvisensis*; therefore only a subset of all stations trawled by DAFF during the spring 2014 and autumn 2015 cruises were selected for analysis. Stations used in this study were spatially matched between species in order to minimize the spatial variation in the ambient food environment during comparative dietary analyses (Fig. 2.2). The selection criteria were two-fold. Mixed trawls (i.e. where both species were caught) were preferentially selected in order to maximize the likelihood that *L. hectoris* and *M. walvisensis* had been exposed to the same food environment. When the first selection criteria was exhausted, single species trawls consisting either of *L. hectoris* or *M. walvisensis* were selected and preference was given to those that were in close proximity to one another (Fig 2.2). For a summary of stations selected and number of samples used per species and per analysis, refer to Table 2.1.

[2] **The Department of Agriculture, Forestry and Fisheries** (Branch: Fisheries) conducted these 2014 and 2015 surveys on-board a commercial industry vessel, the deep-sea stern trawler *MFV Compass Challenger*, which was hired for this purpose and worked according to the standard scientific survey protocol, see Coetzee *et al.* [1] for an example of the survey techniques used in the Benguela current.

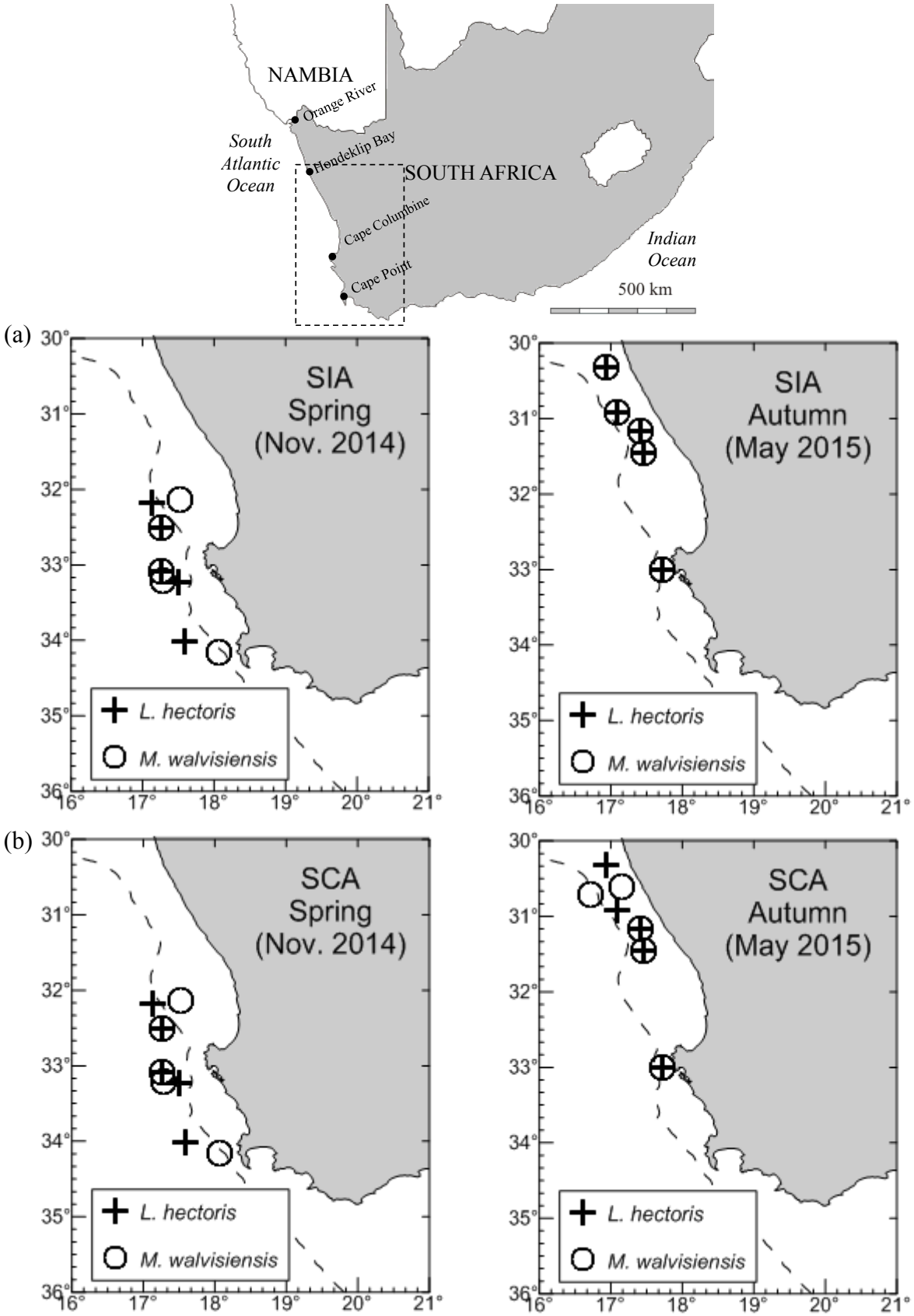


Fig. 2.2. Stations sampled for *Lampanyctodes hectoris* (n=5) and *Maurolicus walvisensis* (n=5) used in (a) stable isotope analysis (SIA) and (b) stomach content analysis (SCA), during the spring 2014 and autumn 2015 pelagic cruises. ⊕ Symbol represents samples of *L. hectoris* and *M. walvisensis* taken from the same (mixed) trawl.

Table 2.1. Details of stations sampled for stable isotope and stomach content analyses during the spring 2014 and autumn 2015 cruises are shown, with the respective sample sizes (n) of *Lampanyctodes hectoris* (*Lh*) and *Maurolicus walvisensis* (*Mw*) used for each analysis.

Station	Stable Isotope Analysis		Stomach Content Analysis		Latitude (°S)	Longitude (°E)	Depth (m)	Date	Time (start)
Spring-14	*Lh*	*Mw*	*Lh*	*Mw*					
C00593	5	---	10	---	-32.1783	17.1296	35	26-Oct	1:47
C00594	---	5	---	10	-32.137	17.5227	106	26-Oct	10:07
C00607	5	5	10	10	-32.5141	17.2694	32	27-Oct	16:42
C00609	5	5	10	10	-33.0785	17.2635	41	27-Oct	20:01
C00614	---	5	---	10	-33.2129	17.2812	80	28-Oct	6:27
C00616	5	---	10	---	-33.2417	17.5041	134	28-Oct	16:03
C00626	5	---	10	---	-34.0191	17.5858	17	29-Oct	21:18
C00627	---	5	---	10	-34.1613	18.0773	102	30-Oct	5:55
Autumn-15									
C00842	5	5	10	---	-30.323	16.9403	93	25-May	21:18
C00845	---	---	---	10	-30.7205	16.7155	208	26-May	7:21
C00846	---	---	---	10	-30.6034	17.1476	106	26-May	13:43
C00852	5	5	10	---	-30.913	17.09	182	27-May	10:47
C00854	5	5	10	10	-31.1597	17.4065	60	27-May	20:41
C00859	5	5	7	10	-31.4545	17.454	145	28-May	9:33
C00888	5	5	10	10	-33.0069	17.7183	147	02-Jun	11:09
Total (n)	**50**	**50**	**97**	**100**					

2.2 Stable isotope analysis

Both *L. hectoris* and *M. walvisensis* possess high lipid concentrations and samples were therefore lipid-corrected to standardize samples for inter- and intra-specific comparisons [39]. However, chemical extraction can also alter $\delta^{15}N$ signals [67]. Samples were therefore analyzed in duplicate to derive accurate $\delta^{15}N$ (non-extracted) and $\delta^{13}C$ (extracted) values (Fig. 2.3), as suggested by the literature [51,52]. Five individual fish per species that were visibly intact and in good condition were selected from the first order subsampling of the chosen stations (Fig. 2.1; Table 2.1). These fish were then thawed. Given the potential for post-thaw degradation of prey, the stomach content of these samples was not used for dietary analyses. Fish were measured to the nearest 0.1mm standard length (SL), here defined as the length from the tip of the premaxilla to the posterior end of the vertebral column, and large

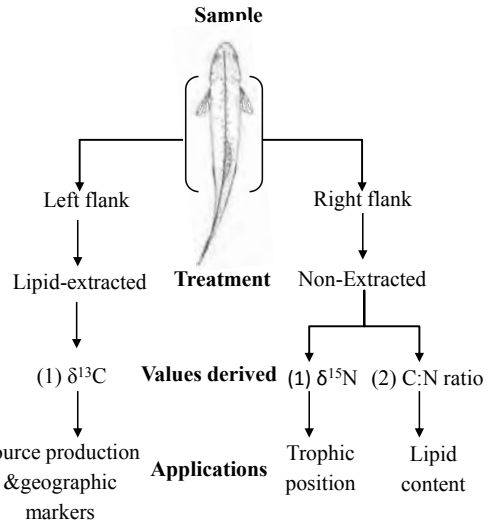


Fig. 2.3. Methodological processes used in stable isotope analysis, as well as the derived values and their uses in this study.

scales were removed. For consistency, white muscle tissue was removed from similar body regions for all specimens. The left flank of the sample was excised for chemical lipid extraction using the Folch method [69] (Fig. 2.3). I immersed the tissue in a 2:1 ratio of chloroform: methanol, with a solvent volume approximately five times that of the sample volume. Lipid extracted samples were then manually agitated for 30 seconds and left undisturbed for a 24 hour period, after which I carefully removed the separated lipid layer from the mixture. I then rinsed and filtered the samples several times with a 0.88% NaCl solution to remove residual lipid content and chloroform-methanol solvent. Tissues removed from the right flank of the sample were not exposed to chemical lipid extraction (i.e. non-extracted), but were rinsed with the NaCl solution for standardization (Fig. 2.3).

I dried all lipid-extracted and non-extracted samples in glass vials at 50˚C for 48 hours and then homogenized individual dried samples with a pestle and mortar. I then weighed aliquots of each sample (0.401-0.498 μg) into tin capsules for analysis. All equipment was cleaned with ethanol after processing each sample. These aliquots (n=100 per species) were analyzed for $\delta^{13}C$, $\delta^{15}N$, % carbon, and % nitrogen by the professional staff at the Archeology Stable Isotope Laboratory at the University of Cape Town. The analytical precision of the instrument was 0.04 ‰ for $\delta^{15}N$ and 0.10 ‰ for $\delta^{13}C$. All carbon to nitrogen ratios (C:N) were reported as uncorrected percent weight calculations and were used as a proxy for lipid content in animal tissues. Stable isotope signatures are expressed in terms of delta (δ) as parts per thousand (‰) differences relative to a known standard, according to the following equation [80]:

$$\delta X = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \quad (1)$$

Where X is the heavy isotope in question (^{13}C or ^{15}N) and R is the ratio of the heavy to light isotopes (i.e. $^{13}C/^{12}C$) of the sample, which is compared against the isotopic ratio of known standards. International standards routinely used by the UCT Archeology Laboratory include Vienna Pee Dee Belamnite for carbon and atmospheric N for nitrogen [51].

2.3 Stomach content analysis

Ten individual fish per species that appeared intact and in good condition were selected from the first order sub-sampling of chosen stations (Fig. 2.1; Table 2.1). Fish were measured to

the nearest 0.1mm SL, and their wet body mass weighed to the nearest 0.001g. For an index of gape, the upper jaw was measured from the tip of the premaxilla to the posterior end of the maxilla to the nearest 0.1 mm. I removed stomachs from the abdominal cavities of partially frozen samples, which were then placed in a 10% formalin solution, neutrally buffered with sodium phosphate, to preserve ingested material. The contents of the esophagus and intestines were not included in these analyses. Excised stomachs were weighed to the nearest 0.001g and the stomach contents per sample were flushed into a petri-dish and examined under a dissecting microscope offering continuous magnification from 0.5 to 3.5x.

Emptied stomachs were re-weighed and the weight of the stomach content was calculated by subtracting the weight of the stomach lining from that of the full stomach. Feeding intensity (%FI) was expressed as a percentage of the wet weight of the stomach content to the total weight of fish [67]. Empty stomachs were documented, though not included in most analyses. The state of prey digestion was classified either as (1) intact and undigested material; (2) moderately digested; (3) unidentifiable digested matter; or (4) completely digested content, consisting mostly of the mucosal lining. Prey items were identified to the lowest possible taxon and, in general, were limited to a family or genus level due to the digested state of most prey items. The eyepiece of the microscope was fitted with a graticule, calibrated at all magnifications, which allowed for total length (µm), prosome length (µm), and prosome width (µm) measurements of ingested prey items to be made, where possible. Smallest measurement resolution achieved using a dissecting microscope was 0.022mm.

To analyze diet composition, the occurrence, numerical frequency, and the prey size of all individuals of each taxon within the stomach contents were recorded. These observations were then used to calculate the nutritional value of each prey category in terms of dietary carbon, which is thought to provide a more representative measure of dietary importance [73]. As such, individual prey items were converted to dry mass (µg) and then to carbon content (µg) using literature derived total length-to-mass and mass-to-carbon relationships (Table 2.2). The carbon content of individual prey was then summed across each taxon present in the sample. When prey items were partly digested or not intact, literature-derived regressions (Table 2.2) were used to convert prosome width or prosome length to the total length for the prey in question.

Table 2.2. Equations used to estimate the dry weight (DW) and carbon content (C) of identifiable zooplankton ingested by *Lampanyctodes hectoris* and *Maurolicus walvisensis*. Dry weight and dietary carbon equations are in µg except where indicated otherwise. Morphometric regressions between the prosome width (PW) and length of prey, either prosome length (PL) or total length (TL) used in this study are shown. Length measurements are in µm.

Taxon		Length to dry weight (µg) regression	Dry weight (µg) to carbon content (µg)	Regressions
Crustacea				
Euphausiacea		$DW\ (mg) = 0.0012\ TL(mm)^{3.16}$ [d]	$C(mg) = 0.424\ DW$ [d]	$TL = 4.2864(PW) - 204.79$ [e]
Amphipoda		$DW\ (mg) = 0.005\ TL(mm)^{2.311}$ [b]	$C(mg) = 0.370\ DW$ [c]	$TL = 1.4089(PW) + 476.78$ [e]
Crustacean larvae		$DW = 3.946\ TL(mm)^{2.436}$ [d]	$C = 0.424\ DW$ [d]	
Copepoda				
Calanoida	*Calanus* sp.	$\ln(DW) = 2.74\ \ln(PL) - 16.41$ [a]	$C = 0.424\ DW$ [d]	$PL = 1.0905\ (PW) + 1120.2$ [e]
	Candacia sp.	$\ln(DW) = 2.74\ \ln(PL) - 16.41$ [a]	$C = 0.424\ DW$ [d]	$PL = 2.2236\ (PW) + 163.2$ [e]
Poecilostomatoida		$\ln(DW) = 1.96\ \ln(PL) - 11.64$ [a]	$C = 0.424\ DW$ [d]	$PL = 1.2138\ (PW) + 192.57$ [e]
Harpacticoida		$\ln(DW) = 1.96\ \ln(PL) - 11.65$ [a]	$C = 0.424\ DW$ [d]	$PL = 5.246\ (PW) + 3.5436$ [e]
Mollusca				
Squid larvae		$DW = 0.0001\ TL^{3.582}$ [b, f]	$C = 0.38\ DW$ [c]	
Gastropod & bivalve larvae		$DW = 47.386\ TL(mm)^{3.663}$ [b]	$C = 0.424\ DW$ [d]	
Fish eggs		$DW = 0.093\ VOL(mm^3) + 0.0012$ [d]	$C = 0.457\ DW$ [d]	

[a] Chisholm & Roff [81]
[b] James [82]
[c] Espinoza & Bertrand [83]
[d] van der Lingen [84]
[e] Mketsu [85]
[f] TL estimated from the length of the squid pen

2.4 Trophic position estimates

Non-extracted $\delta^{15}N$ values from stable isotope analyses (SIA) were used to calculate the relative trophic position for individuals of *L. hectoris* and *M. walvisensis* using the equation [62]:

$$TP_{SIA} = \lambda + \left({}^{\delta^{15}N_i + \delta^{15}N_{ref}}/_{3.4‰} \right) \quad (2)$$

where $\delta^{15}N_i$ is the isotopic measurement of individual fish and 3.4‰ is the average trophic enrichment between muscle of fish and their food [51]. $\delta^{15}N_{ref}$ represents the average $\delta^{15}N$ signal which characterizes the base of the food-web and λ is the trophic level of the reference organism(s) used for the baseline. Although $\delta^{15}N$ baselines were not analyzed due to the time and scope constraints of the study, two $\delta^{15}N$ baseline references were derived from the literature. The first is the intertidal mussel *Mytilus galloprovincialis* (8.35‰) sampled from St. Helena Bay in 2006 [65]. Filter feeders like *M. galloprovincialis*, which occur in depths less than 40m, have an estimated trophic position (λ) of 2.4 [49].The second baseline estimate was derived from mixed phytoplankton (1.2‰ and λ = 1) sampled at the shelf edge near Walvis Bay in the northern Benguela upwelling system in 2009 [64]. Post [57] recommends that the reference organisms used as a baseline estimate of the food web should (1) share the same habitat as the study species and (2) integrate the isotopic signature of the food web at a time scale large enough to minimize the ejects of short-term variation. Consequently, the spatial and/or temporal variation of $\delta^{15}N$ in the marine systems is of concern and both baseline estimates used in this study are likely to introduce error in the derived trophic positions.

Data collected during stomach content analyses (SCA) were used also to calculate the trophic position of individual fish for *L. hectoris* and *M. walvisensis* using the following equation [86]:

$$TP_{SCA} = 1 + \left(\sum_{i=1}^{n} P_i \times TP_i \right) \quad (3)$$

Where P_i reflects the proportion of each prey category, here calculated in terms of prey contribution to dietary carbon, and TP_i is the trophic position of the *i*th prey item. Trophic positions for respective prey categories were taken from several published accounts [10,20,64].

2.5 Data analysis

Statistical tests were conducted using Rstudio software [87], while diet composition was analyzed with multivariate analytical techniques, using PRIMER software with PERMANOVA (Permutational Multivariate Analysis of Variance)[88, 89]. All two-factor analyses of variance tests (ANOVA) used in this study were followed by *post-hoc* Tukey pair-wise comparison tests to determine where significant differences lay. All parametric tests used in this study were checked for normalcy and homogeneity of variances. Statistical significance was determined when $p<0.05$. All values are reported with the mean ± 1 standard error (SE), unless otherwise indicated. Regression analysis fitted a model (linear or non-linear) to the data. R^2 and *p*-values are shown; the *p*-value indicates the significance of the relationship and R^2 indicates how well the data fits the regression model used.

2.5.1 Morphometric comparisons

Size is known to influence the diet (and hence stable isotope values) of fishes and it is often cited as a possible explanation for observed inter-specific differences. Consequently, the morphometric data (standard lengths and gape size) was pooled from both analyses to examine inter- and intra-specific differences in fish length and gape size. A one-way ANOVA was conducted to compare mean SL between species, followed by one-way ANOVAs within each species to determine if fish sampled in spring 2014 and autumn 2015 differed significantly in their mean SLs. Potential size differences were of interest as they could confound any interspecific or seasonal effects detected. Furthermore, gape size is an important constraint on the upper-limit of prey-size ingested and often provided as a possible explanation for correlations seen between prey type and predator size. Consequently, the relationship between gape size and SL was examined using regression analysis, followed by ANCOVA (with control for body size) to detect for inter-specific differences in gape.

2.5.2 Stable isotope analysis

Firstly, C:N ratios were used as a proxy for lipid content and these values were compared against the recommended 3.5 ratio [51] using one-sample t-tests to validate that *L. hectoris* and *M. walvisensis* required lipid correction. The effect of chemical lipid extraction on $\delta^{13}C$, $\delta^{15}N$, and C:N ratios was also examined. Differences between extracted and non-extracted bulk values

were determined using paired t-tests (p<0.05) for (1) *L. hectoris*, (2) *M. walvisensis*, and (2) both species pooled. A Holm test was performed to reduce the probability of committing type I errors resulting from multiple comparisons [67].

Stable isotope values of *L. hectoris* and *M. walvisensis* were examined for interspecific, seasonal, and size-related variability in their trophic positions ($\delta^{15}N$) and source production ($\delta^{13}C$). Inter-specific and seasonal variations in $\delta^{13}C$, $\delta^{15}N$, and C:N were tested using a two-way ANOVA design, each factor consisting of two levels: species (*L. hectoris* vs. *M. walvisensis*) and season (spring 2014 vs. autumn 2015). The relationship between isotopic values and SL were explored using linear regression analysis for *L. hectoris* and *M. walvisensis*, separated by season.

2.5.3 Stomach content analysis

For each prey category, three main diet indices were calculated: frequency of occurrence as proportions of predator stomachs containing said prey (%F), numerical importance as the proportion of total abundance (%N), and nutritional importance as the proportion of total dietary carbon (%C). To facilitate analysis and ecological interpretation, prey taxa were grouped by functional group based on known taxonomic relations and ecological traits (i.e. meso- vs. macrozooplankton). Cumulative prey curves were used to assess whether or not the stomachs sampled from *L. hectoris* and *M. walvisensis* were sufficient to describe diet diversity and breadth. For nutritional studies on fish, the diversity of ingested prey is of interest as it can reflect the general feeding strategies of target species. Differences in the average dietary breadth of fishes were therefore examined using the Shannon-Wiener index ($H' \log_e$) as an index of dietary diversity and compared with a two-way ANOVA for species and season,

To test for differences in diet composition, sample by prey taxa matrices were generated for prey occurrence, numerical frequency, and carbon content data. As recommended by Clarke & Gorley [89], all matrices were pre-treated with a 4th root transformation in order to down-weigh the contribution of quantitatively dominant species; thus taking into account the importance of intermediate and rare species. Diet composition was compared among samples using the Bray-Curtis measure of (dis)similarity and inter-specific differences were summarized using MDS plots. PERMANOVA main effects were used to test for significant differences in diet composition between *L. hectoris* and *M. walvisensis*, as well as for seasonal effects. When dif-

ferences or interactions were significant (p<0.05), pairwise permutation tests were conducted to see where these differences lay (i.e. which groups differ significantly from one another). Finally, a similarity of percentages analysis (SIMPER, part of the PRIMER software package) was used to assess which prey items contributed most to the differences found between groups. Diet composition was similarly compared by the time at which samples were collected, using three categories: twilight (TW=05:20-07:30 and 18:30-20:30), daylight (D=07:30-18:30), and night (N=20:30-05:30).

Feeding periodicity was also of interest as mesopelagic fishes undertake vertical diel migrations corresponding to the vertical movement of zooplanktonic prey. Asynchronous feeding cycles may therefore facilitate resource partitioning within the mesopelagic assemblage. Feeding chronologies were determined by comparing both (1) the state of prey digestion and (2) feeding intensity versus time of capture, using a two-way ANOVA design by species and time. However, a common dilemma associated with chronoecological studies is that the stomach content of fishes may not necessarily correspond to feeding activity around the time that these were caught [90,91]. To validate any chorological differences in feeding intensity, the digestion state of prey was also interpreted [92,93] (Fig. 2.3). Prey in digestion states 1 and 2 were likely from "recent" feeding events, whereas prey in states 3 and 4 were from "previous" feedings [92,93].

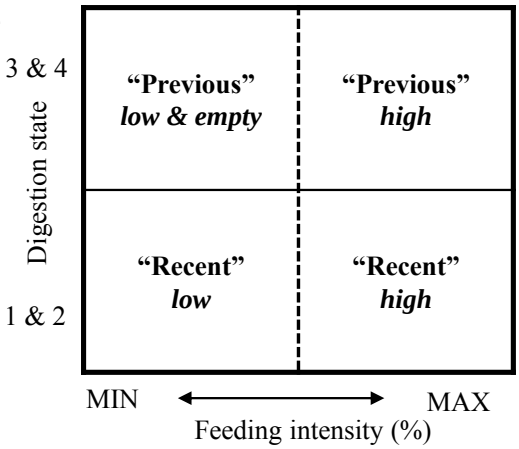


Fig. 2.4. Schematic diagram showing the prey digestion state (categorical) and percent feeding intensity (continuous) used to interpret feeding periodicities of mesopelagic fishes, figure modified from Olson & Galván-Magaña [93].

The validity of this approach depends on several assumptions, (1) that the overall appearance of the stomach content (i.e. state of digestion) is unaffected by differences in the amounts and types of food ingested; (2) prey digestion ceased once fish were caught; and (3) that the state of digestion increases with time since ingestion. The latter is a standard assumption used to evaluate feeding periodicity [91]. Enzymatic reactions in the digestive tract are essentially exponential processes; therefore the rate of digestion and food evacuation often proceeds at an exponential rate in teleost fish [91]. However, the reported mathematical models fitted to the data in gas-

tric digestion and evacuation studies in marine systems have varied extensively, including but not limited to linear, exponential, and polynomial regressions [91]. Although it cannot be assumed that the rate of digestion is exponential for all fish species [91], including mesopelagic fishes, it nevertheless has been shown to increase in a predictable and constant manner with time. Though gastric digestion is a chemical process that continues after the death of the fish [90], the act of blast freezing followed by thawing in neutrally buffered formalin is thought to preserve stomach contents from post-capture digestion. Therefore the second assumption appears reasonable, leaving only the first as a major assumption to be considered when interpreting the results.

Given that fish size, in terms of length and gape, is thought to influence the size range of ingested prey, variation in prey size was investigated. The mean size of ingested prey (mm) was calculated for individuals within each species, which were then examined for interspecific and seasonal differences using a two-way ANOVA. Given the strong correlation detected between SL and gape, prey size was plotted solely against the standard length of *L. hectoris* and *M. walvisensis*, and these relationships were examined using non-linear regression analysis.

2.5.4 Trophic position estimates

Diet derived and SIA derived trophic positions for *L. hectoris* and *M. walvisensis* were compared using a two-way ANOVA design, testing for differences by species and by method. Trophic positions were also compared between species and season using a two-way ANOVA design. In addition, the relationship between trophic position and SL was examined using linear regression analysis, followed by ANCOVA.

Chapter 3: Results

3.1 Morphometric comparisons

On the whole, lanternfish *Lampanyctodes hectoris* were significantly larger than lightfish *Maurolicus walvisensis*, with average SLs of 54.8mm (SE = ±0.8, n=150) and 37.7mm (±0.6, n=147), respectively ($F_{(1,295)}$ = 383.70, p<0.001). No significant difference between seasons in SL was detected for *L. hectoris* (p=0.092; Fig. 3.1). By contrast, *M. walvisensis* sampled in the autumn were significantly larger than those sampled in the spring, with an average SL of 40.9mm (±0.5, n=75) and 37.7mm (±0.6, n=75), respectively ($F_{(1,148)}$ = 16.68, p<0.001; Fig. 3.1).

A strong positive relationship was detected between gape size and standard length for both species (Fig. 3.2), and *L. hectoris* and *M. walvisensis* demonstrated considerable differences in gape. ANCOVA revealed that the rate of change in mouth gape relative to SL was different between the two species ($F_{(1,193)}$ = 475.08, p<0.001); and that overall gape size also significantly differed between *L. hectoris* and *M. walvisensis* ($F_{(1,193)}$ = 2863.97, p <0.001). Consequently, *L. hectoris* individuals had a larger gape than those of *M. walvisensis* of equivalent size, and gape size increased more rapidly with increasing SL for the former.

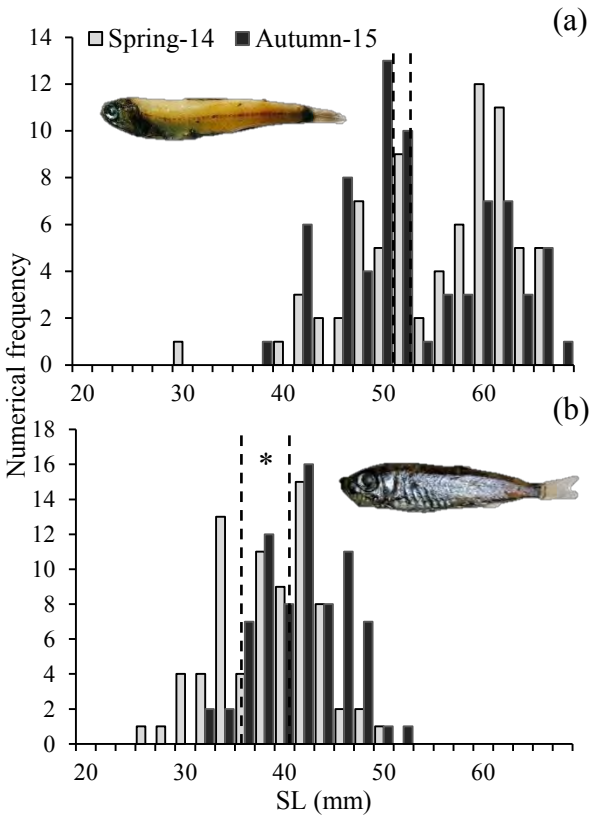


Fig. 3.1. Standard length (SL mm) frequency distributions by season sampled for (a) *Lampanyctodes hectoris* (spring n=75, autumn n=72) and (b) *Maurolicus walvisensis* (spring n=75, autumn n=75). Dashed lines denote the mean SL by season and the asterisk denotes statistically different values (p<0.05).

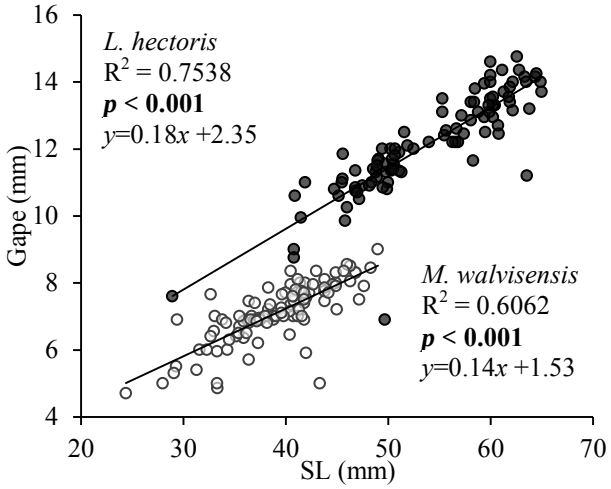


Fig. 3.2. Relative gape size (mm) versus standard length (mm) for individual fish of *Lampanyctodes hectoris* (n=97) and *Maurolicus walvisensis* (n=100), pooled across spring 2014 and autumn 2015 cruises. Linear regressions, R^2, and p-values are shown, significant values (p<0.05) are in bold.

3.2 Stable isotope analysis

3.2.1 Effects of lipid extraction

Non-extracted C:N ratios were found to be significantly higher than the recommended 3.5 for both *L. hectoris* ($t_{(98)}$= -9.1272, p<0.001) and *M. walvisensis* ($t_{(98)}$ = -9.1272, p<0.001), validating the need for lipid correction in this study (for mean isotopic values see Table 3.1). The $\delta^{13}C$ and $\delta^{15}N$ signals of lipid-extracted samples were significantly and consistently higher relative to non-extracted samples in every pairwise case (p<0.001, Table 3.1). Furthermore, muscle C:N ratios from lipid-extracted samples were significantly and consistently lower than non-extracted samples in both species (p<0.001, Table 3.1). These results indicated that chemical lipid extraction was an effective method for removing lipid bias from bulk $\delta^{13}C$ for these species. However, analyzing samples in duplicate was necessary as lipid extraction was also found to significantly alter $\delta^{15}N$ measures.

Table 3.1. The effect of chemical lipid extraction using the Folch method [69] on the $\delta^{13}C$, $\delta^{15}N$, and C:N values of individual samples (paired t-tests) for *Lampanyctodes hectoris* (n=50) and *Maurolicus walvisensis* (n=50). Mean values and differences (± SE) are given. Significant p-values (p<0.05) are shown in bold.

Response variable	Species	df	t-value	*p*	e tr cte	non-extracted	(extracted-nonextracted)
$\delta^{15}N$	Pooled	99	20.04	**<0.001**	13.72 (0.07) ‰	13.05 (0.08) ‰	0.67 (0.034) ‰
	L. hectoris	49	30.91	**<0.001**	14.18 (0.05) ‰	13.57 (0.04) ‰	0.61 (0.020) ‰
	M. walvisensis	49	11.578	**<0.001**	13.27 (0.10) ‰	12.54 (0.11) ‰	0.73 (0.063) ‰
$\delta^{13}C$	Pooled	99	19.73	**<0.001**	-17.08 (0.07) ‰	-18.49 (0.08) ‰	1.40 (0.071)‰
	L. hectoris	49	19.81	**<0.001**	-16.97 (0.09) ‰	-18.51 (0.12) ‰	1.46 (0.11) ‰
	M. walvisensis	49	10.838	**<0.001**	-17.20 (0.11) ‰	-18.46 (0.09) ‰	1.2‰ (0.14) ‰
C:N†	Pooled	99	-14.4	**<0.001**	3.53 (0.03)	4.85(0.11)	-1.33 (0.092)
	L. hectoris	49	-15.35	**<0.001**	3.67 (0.05)	5.12 (0.15)	-1.46 (0.11)
	M. walvisensis	49	-9.35	**<0.001**	3.39 (0.02)	4.59 (0.15)	-1.20 (0.14)

†C:N ratios were log transformed to meet t-test assumptions of normality; mean ± SE values were derived from untransformed data.

3.2.2 Inter-specific & seasonal variation in isotopic composition

The mean $\delta^{13}C$ (lipid-extracted) and $\delta^{15}N$ (non-extracted) isotopic values of *Lampanyctodes hectoris* and *M. walvisensis* were compared between species and season using a two-way ANOVA, the results of which are shown in Table. 3.2. $\delta^{15}N$ was significantly and consistently higher for *L. hectoris* when compared with M. *walvisensis,* irrespective of season (p<0.001; Fig. 3.3a). The difference in $\delta^{15}N$ between species was 1.02‰ (±0.067, n=100), which constitutes a mean difference of nearly third of one trophic position (assuming a trophic enrichment of +3.4‰

per TP). As a result, *L. hectoris* occupied a higher relative trophic position than *M. walvisensis* based on their $\delta^{15}N$ signals. Lipid-extracted $\delta^{13}C$ values of *L. hectoris* and *M. walvisensis* were compared (Fig. 3.3b). *Post-hoc* tests revealed that *L. hectoris* differed significantly in its $\delta^{13}C$ signals ($p<0.001$) from *M. walvisensis* in both cruise seasons; however, the inter-specific difference was most pronounced among individuals sampled in the spring. These differences may, however, be a consequence of size and interpreting the biological significance of these results must be done with caution (section 3.1).

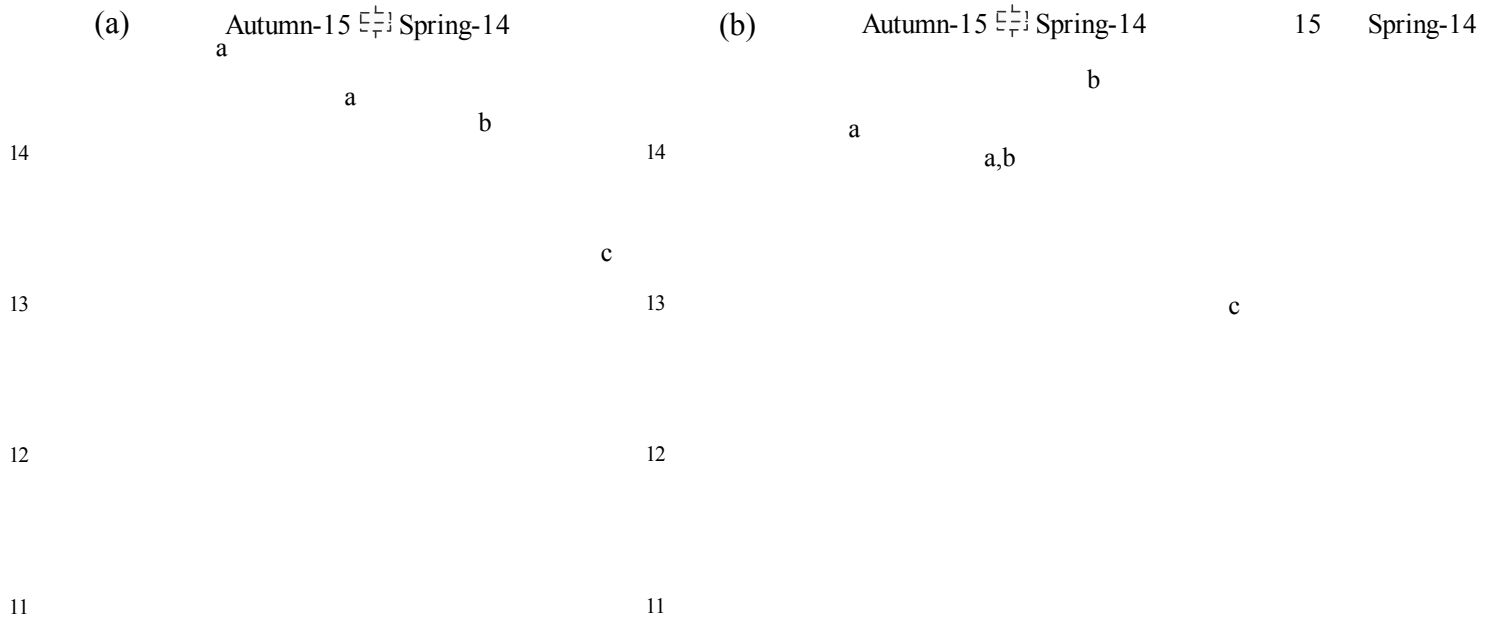


Fig. 3.3. Boxplots of (a) non-extracted $\delta^{15}N$ values and (b) lipid-extracted $\delta^{13}C$ values by season, spring 2014 and autumn 2015, and by species, *Lampanyctodes hectoris* (spring n=25, autumn n=25) and *Maurolicus walvisensis* (spring n=25, autumn n=25). Letters represent the results of Tukey's *post-hoc* comparisons of group means, shared letters indicate values that are not significantly different ($p > 0.05$). The median, interquartile range, min and max values (whiskers), and (•) outliers, as determined by R software, are shown.

Significant interactions between species and season were detected for both isotopic values ($\delta^{15}N$ and $\delta^{13}C$; $p<0.001$, Table 3.2). Outcomes of *post-hoc* Tukey tests are shown in Fig. 3.3. These results indicate that only *M. walvisensis* differed significantly in its $\delta^{15}N$ and $\delta^{13}C$ values by season ($p<0.001$); both being higher in the autumn than the spring (Fig. 3.3), which likewise may be a consequence of size differences detected between seasons for this species (see section 3.1). Lipid content was similarly examined for inter-specific and seasonal variation and these results are shown in Appendix C.

Table 3.2. The results of $\delta^{13}C$ and $\delta^{15}N$ analyses compared by season (spring 2014 and autumn 2015) and by species, *Lampanyctodes hectoris* (spring n=25, autumn n=25) and *Maurolicus walvisensis* (spring n=25, autumn n=25), using a two-way ANOVA design. Significant *p*-values ($p < 0.05$) are shown in bold.

Source of variation		Degrees of freedom	Sum of squares	Mean square	F ratio	P-value
$\delta^{15}N$	Species	1	26.204	26.204	137.45	**<0.001**
	Season	1	11.404	11.404	59.82	**<0.001**
	Season*Species	1	4.314	4.314	22.63	**<0.001**
$\delta^{13}C$	Species	1	1.386	1.386	5.239	**0.024**
	Season	1	6.404	6.404	24.202	**<0.001**
	Season*Species	1	16.118	16.118	60.918	**<0.001**

Isotopic biplots ($\delta^{13}C$ vs. $\delta^{15}N$) of *L. hectoris* and *M. walvisensis* separated by season were examined and are shown in Fig. 3.4. Although some degree of overlap exists between *L. hectoris* and *M. walvisensis*, particularly in $\delta^{13}C$, the two species were separated in isospace by their $\delta^{15}N$ values (Fig. 3.4). A significant difference between the spring and autumn cruises was observed for *M. walvisensis,* with clear separation by season in both axes ($\delta^{13}C$ and $\delta^{15}N$). Though the seasonal variation was consistent with the seasonal differences detected in $\delta^{13}C$ and $\delta^{15}N$ for *M. walvisensis* (see above), the observed shift from the lower left quadrant (spring 2014) to the upper right (autumn 2015) could again be a consequence of larger fish sampled in the autumn versus the spring for this species (see section 3.1).

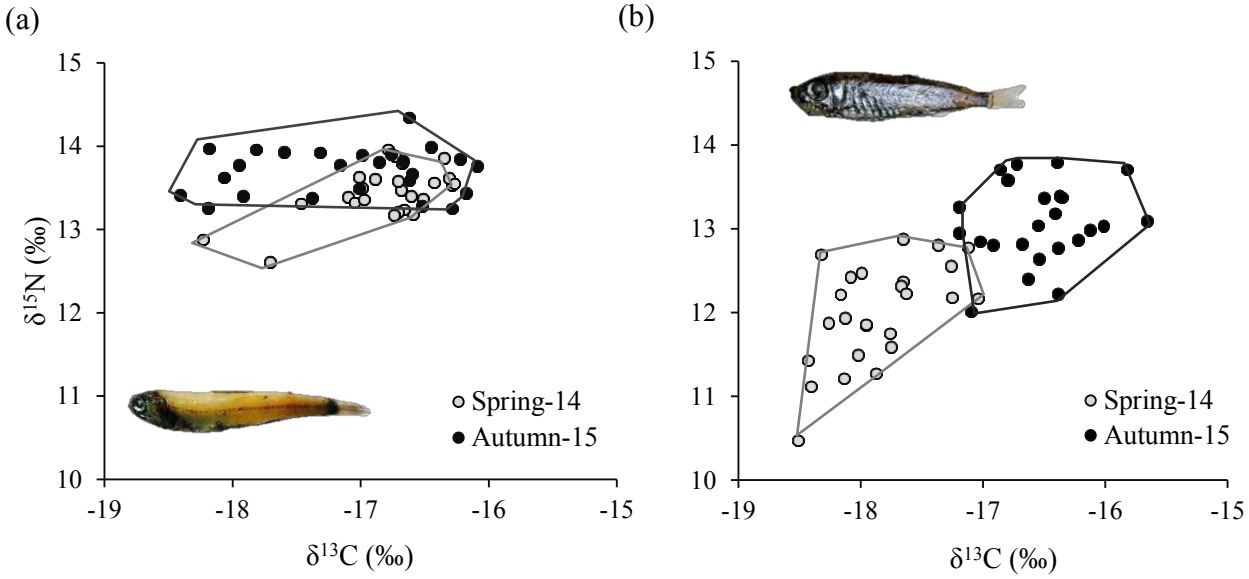


Fig. 3.4. Biplots comparing $\delta^{13}C$ and $\delta^{15}N$ values by season (spring 2014 and autumn 2015) for (a) *Lampanyctodes hectoris* (spring n=25, autumn n=25) and (b) *Maurolicus walvisensis* (spring n=25, autumn n=25). The polygons reflect the relative isospaces of each species by season.

3.2.3 Size related shifts in stable isotope ratios

Size related shifts were investigated for *L. hectoris* (Fig. 3.5) and *M. walvisensis* (Fig. 3.6) by examining the relationships between standard length and isotopic values ($\delta^{15}N$ and $\delta^{13}C$) for each season sampled. Since the SL-gape relationship was robust, SL was the only variable against which the isotopic ratios were plotted. Although the samples of both species showed a trend of increasing $\delta^{15}N$ with increasing size, only the relationship for *L. hectoris* in the spring 2014 cruise was significant (Fig. 3.5; R^2=0.404, p=0.0006). Size effects on $\delta^{13}C$ signals exhibited great variability with season for *L. hectoris*. The spring cruise was characterized by a positive relationship between $\delta^{13}C$ and SL (Fig. 3.5; $R^2 = 0.234$, $p = 0.014$), while a negative relationship was observed in the autumn ($R^2 = 0.357$, $p = 0.0016$). However, no significant relationships between SL and isotopic values ($\delta^{13}C$ and $\delta^{15}N$) were observed for *M. walvisensis*, suggesting opportunistic foraging for this species. For the size-related relationships between SL and C:N ratios for *L. hectoris* and *M. walvisensis*, see Appendix C.

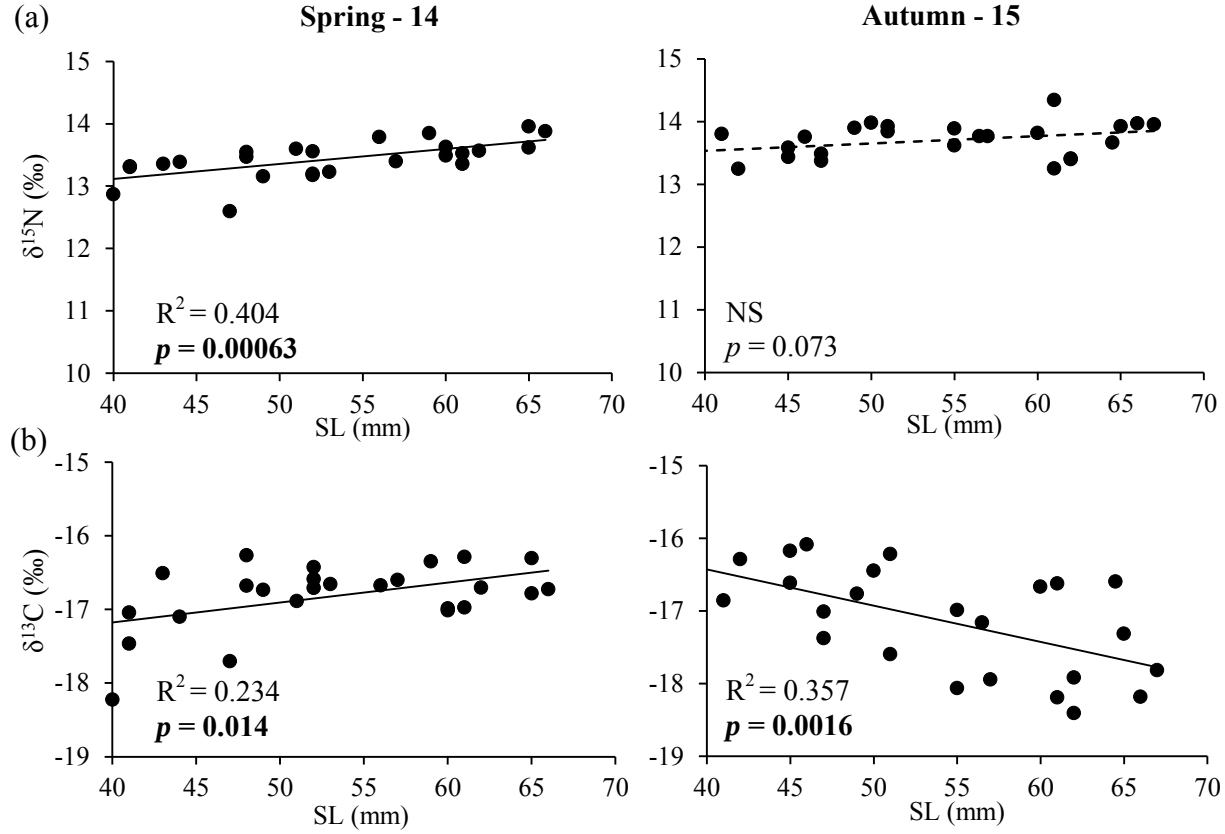


Fig. 3.5. Relationships between standard length (SL) and (a) $\delta^{15}N$ and (b) $\delta^{13}C$ for *Lampanyctodes hectoris* by season: spring 2014 (n=25) and autumn 2015 (n=25). Solid lines indicate significance in linear regressions (p<0.05), dashed lines indicate lack of significance but possible relationship as suggested by low p-values (1<p<0.05). R^2 and p-values shown, significant values (p<0.05) are in bold. Non-significant (NS) relationships are indicated.

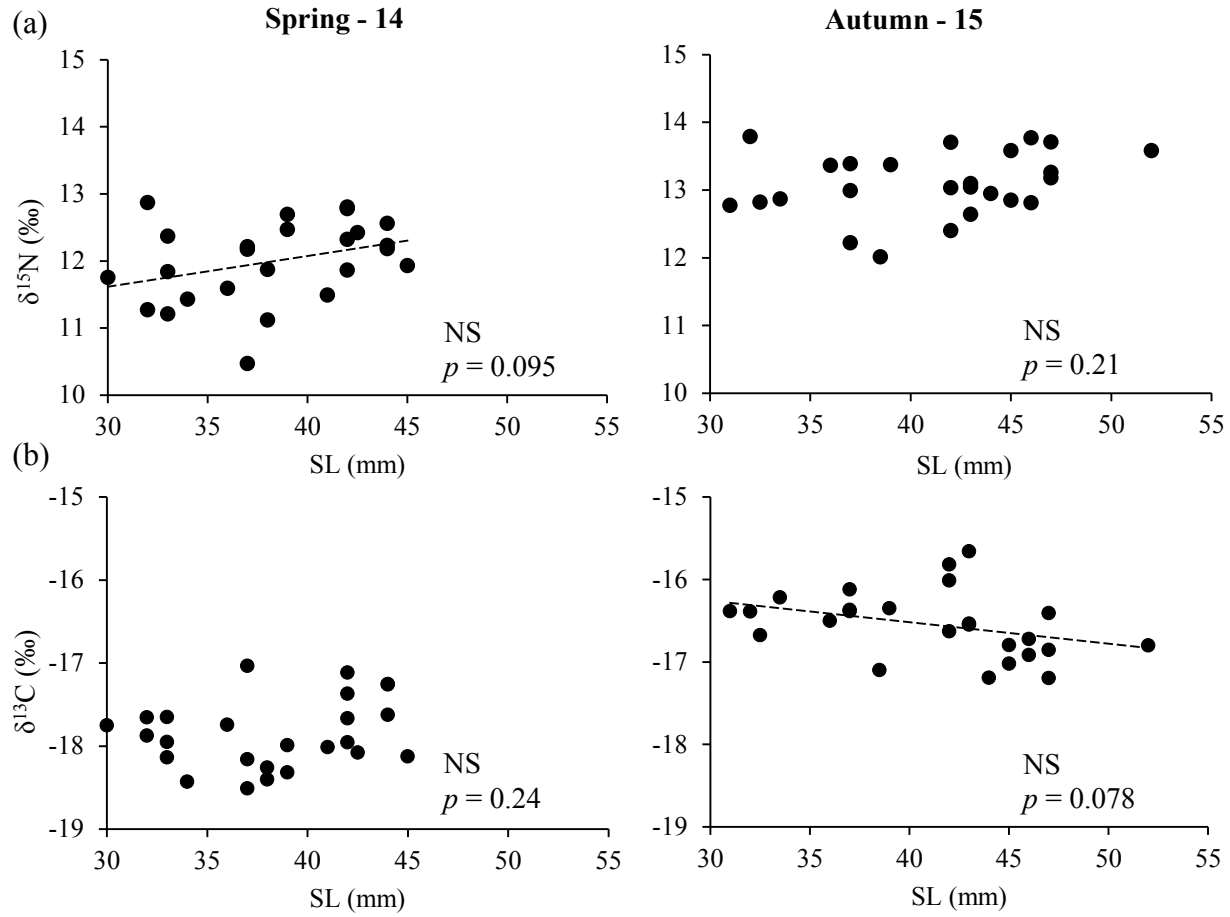


Fig. 3.6. Relationships between standard length (SL) and (a) $\delta^{15}N$ and (b) $\delta^{13}C$ for *Maurolicus walvisensis* by season: spring 2014 (n=25) and autumn 2015 (n=25). Solid lines indicate significance in linear regressions ($p<0.05$), dashed lines indicate lack of significance but possible relationship as suggested by low p-values ($1<p<0.05$). Absence of lines indicates no statistical relationship; non-significant (NS) relationships are indicated and p-values shown.

3.3 Stomach content analysis

3.3.1 Overview of stomach content analysis

Stomach contents were analyzed from a total of 97 fish belonging to *L. hectoris* and 100 belonging to *M. walvisensis* over the spring 2014 and autumn 2015 cruises combined. The frequency of stomachs containing food were greater in the spring than the autumn for both species sampled (Appendix D), but no significant differences were detected by species or season (χ^2=0.619, p = 0.43). Overall, 79.2% of the fish sampled had food in their stomachs, from which 14 different prey types of varying taxonomic resolution were identified (Appendix D). These can be further grouped under the broad prey categories of Amphipoda, Copepoda, Euphausiacea, Mollusca larvae, and Fish eggs.

3.3.2 Diversity of ingested prey

Cumulative prey curves indicate that sample sizes for both species were probably not sufficient to fully describe diet diversity, as none of the curves approach an asymptote (Fig. 3.7). Nevertheless, *M. walvisensis* does appear to have a broader dietary breadth than *L. hectoris* based on the total number of food categories consumed (Fig. 3.7).

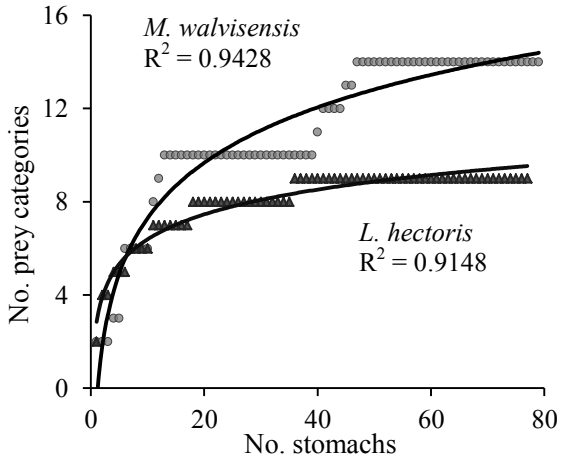


Fig. 3.7. Cumulative prey curves for *Lampanyctodes hectoris* (n=77) and *Maurolicus walvisensis* (n=79), pooled across spring 2014 and autumn 2015 cruises. The lines correspond to log(e) regressions; R^2 values shown.

Shannon-Wiener diversity H' indices were calculated in terms of carbon contribution for prey taxa and were found to differ significantly between the two species ($F_{(1,152)}$ 7.37, p = 0.0074), with the diet composition of *M. walvisensis* (H'=0.30±0.04) being more diverse than *L. hectoris* (H'=0.18±0.03). Here, greater dietary breadth was indicative of generalist feeding behavior for *M. walvisensis*; while *L. hectoris* showed a degree of diet specialization. Though H' did not differ by season overall (p=0.66), the interaction between species and season was of significance ($F_{(1,152)}$ = 12.25, p<0.001) and the results *of post-hoc* Tukey tests are shown in Fig. 3.8. This study found that the dietary breadth significantly decreased from spring to autumn for *M. walvisensis* (p=0.03); but not for *L. hectoris* (p=0.14; Fig. 3.8).

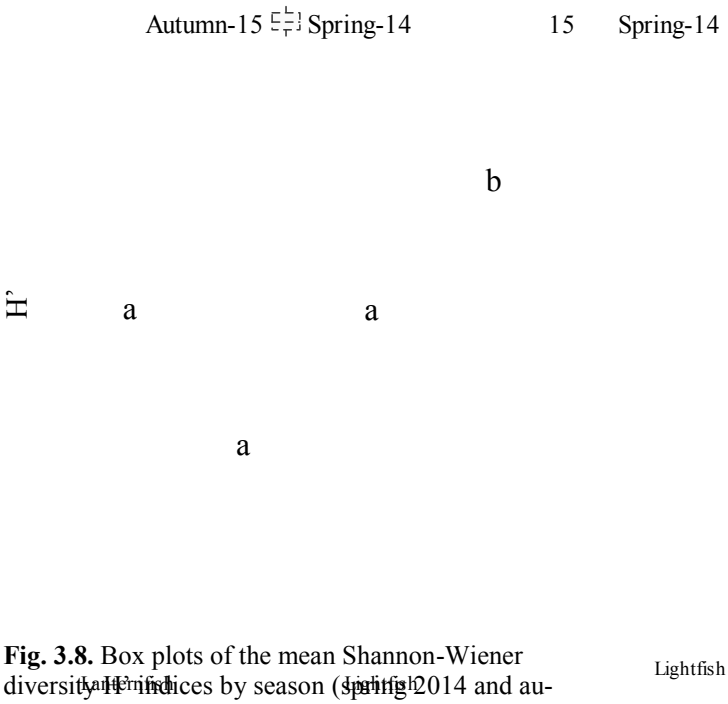


Fig. 3.8. Box plots of the mean Shannon-Wiener diversity H' indices by season (spring 2014 and autumn 2015) for *Lampanyctodes hectoris* (spring n=41, autumn n=36) and *Maurolicus walvisensis* (spring n=47, autumn n=32). Letters represent the results of Tukey's *post-hoc* comparisons of group means, shared letters indicate values that are not significantly different (p >0.05). The median, interquartile range, min and max values (whiskers), and (•) outliers, as determined by R software, are shown.

3.3.3 General diet compositions

Ingested euphausiids and amphipods were characterized by a broad range in their total lengths. To better elucidate differences in their dietary representation by size, they were divided into size classes for subsequent analyses. Amphipods were separated into small (< 2mm) and large (≥ 2mm) size classes and euphausiids were separated into the morphologically distinct adult and larval life stages. The division between size classes for both prey categories were reflected in their frequency distributions (Appendix E).

The diet composition in terms of percent frequency of occurrence (%F), percent numerical frequency (%N), and percent carbon contribution (%C) for *L. hectoris* and *M. walvisensis* was examined (Appendix D) and the results are summarized in Fig. 3.9. Both species are zooplanktivorous, preying on a number of taxa of meso- and macro-zooplankton. Averaged across the spring 2014 and autumn 2015 cruises, the diet of *L. hectoris* (in terms of carbon) consisted of approximately 65% macro-zooplankton and 35% meso-zooplankton, while the diet of *M. walvisensis* consisted of 20% macro-zooplankton and 80% meso-zooplankton. More specifically, copepods were the most frequently occurring (63.6% F) and numerically important (41.5% N) component for *L. hectoris*. However the most important prey for *L. hectoris*, in terms of their dietary carbon, were adult-stage euphausiids (52.5% C). By comparison, copepods, particularly the order Calanoida (*Calanus* sp.), were the most frequent (83.5% F), abundant (64.1% N), and nutritionally important (66.9% C) component of the diet for *M. walvisensis*. Adult-stage euphausiids (17.7% F, 13.7% N, 17.5% C) and small amphipods (48.1% F, 15.8% N, 10.7 % C) were next in importance for this species, however.

Though both size classes of amphipods were consumed by *L. hectoris* (50.6% F) and *M. walvisensis* (51.9% F), large and small amphipods contributed to their respective diets in different proportions (Fig. 3.9). Large amphipods were more important to the diet of *L. hectoris*, while small amphipods were more important for *M. walvisensis* (Appendix D). Although euphausiid larvae and fish eggs were found in 11 – 14% of fish stomachs sampled, their contribution to dietary carbon was negligible for both *L. hectoris* and *M. walvisensis* (<1%; Fig 3.9).

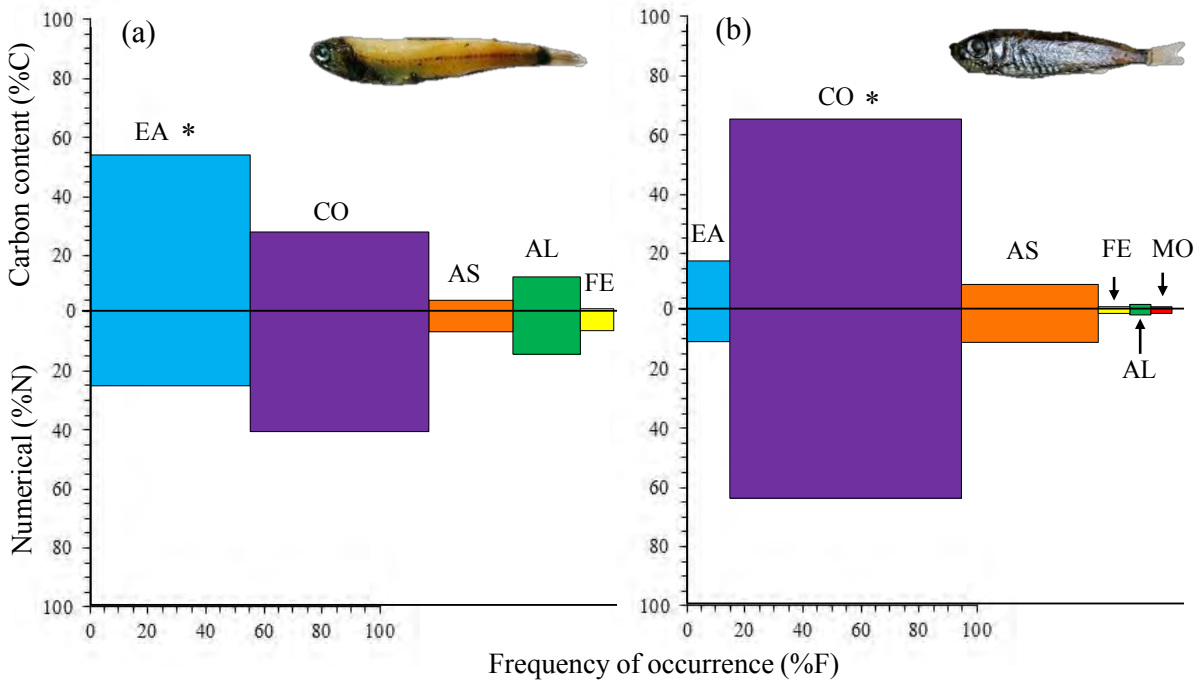


Fig. 3.9. Diet composition of (a) *Lampanyctodes hectoris* (n=77) and (b) *Maurolicus walvisensis* (n=79), pooled across spring 2014 and autumn 2015 cruises. Dietary importance was expressed in terms of percent frequency of occurrence (%F), percent numerical abundance (%N), and percent carbon contribution (%C). Dominant prey items in terms of dietary carbon are indicated with an asterisk. Prey taxa: EA – adult-stage euphausiids, CO – copepods, AS – small (< 2mm) amphipods, AL – large (> 2mm) amphipods, FE- fish eggs, MO – mollusc larvae. Note that the sum of %F across all prey categories can exceed 100%.

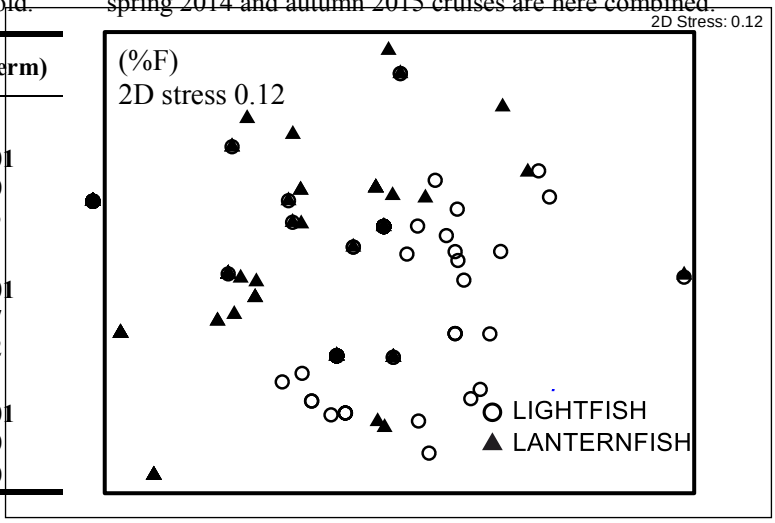
3.3.4 Interspecific diet comparisons

The diets of *L. hectoris* and *M. walvisensis* were compared at the highest possible taxonomic resolution in terms of prey occurrence, numerical abundance, and carbon contribution. Non-metric multidimensional scaling (MDS) was performed and the plot for frequency of prey occurrence shows some degree of separation in diet composition by species (Fig. 3.10). Similarly, PERMANOVA analyses revealed significant differences in diet composition between *L. hectoris* and *M. walvisensis* for all three dietary measures (Table 3.3). SIMPER revealed that the dissimilarity between species was attributable to a greater average abundance of copepods (particularly *Calanus* sp.), small amphipods, and euphausiid larvae contributing to the diet of *M. walvisensis* and, conversely, a greater abundance of adult-stage euphausiids and large amphipods contributing to the diet of *L. hectoris*. These prey categories responsible for driving the difference detected between *L. hectoris* and *M. walvisensis* diets were consistent across all three datasets and diet composition did not differ by season for either species (Table 3.3).

Table 3.3. PERMANOVA results comparing diet composition by season (spring 2014 and autumn 2015) and by species, *Lampanyctodes hectoris* (spring n=41, autumn n=36) and *Maurolicus walvisensis* (spring n=47, autumn n=32), using three different measures of diet: (a) dietary carbon, (b) numerical abundance, and (c) occurrence. Significant *p*-values ($p<0.05$) are in bold.

Source of variation	df	SS	MS	Pseudo-*F*	*P*(perm)
(a) Carbon					
Species	1	35012	35012	14.5	**0.001**
Season	1	3565	3565	1.5	0.20
SpxSe	1	404.4	404.4	0.2	0.93
(b) Numerical					
Species	1	25167	25167	10.5	**0.001**
Season	1	5678.3	5678.3	2.4	0.07
SpxSe	1	1525.6	1525.6	0.6	0.62
(c) Occurrence					
Species	1	25029	25029	11.0	**0.001**
Season	1	4908.6	4908.6	2.2	0.10
SpxSe	1	311.8	311.8	0.1	0.90

Fig. 3.10. MDS plot representing Bray-Curtis similarity for prey data, comparing diets by species (lanternfish *Lampanyctodes hectoris* and lightfish *Maurolicus walvisensis*) in terms of prey occurrence (%F).The plot was determined three-dimensionally, but is presented in two dimensions and the stress value is shown. Note that both spring 2014 and autumn 2015 cruises are here combined.



3.3.5 Feeding periodicity

Stomach contents were similarly analyzed to determine whether time (daylight, twilight, and night) affected diet composition. Statistically, similar prey taxa were consumed by *L. hectoris* and *M. walvisensis* irrespective of the time at which fish were sampled ($p>0.05$). With that said, however, feeding intensity and state of prey digestion were examined for evidence of feeding periodicity within either species. Analysis revealed that feeding intensity did not differ between species ($p=0.54$) or time ($p=0.39$), but the interation between these two variables was of signficance ($F_{(2,191)}=8.21$, $p<0.001$) and the outcomes of *post-hoc* tests are shown in Fig. 3.11a. By contrast, state of digestion differed significantly by species ($F_{(1,191)}=4.43$, $p=0.037$) and time ($F_{(2,191)}=3.62$, $p=0.029$). Nevertheless, these results combined indicate that feeding intensity for *L. hectoris* was at a maximum during twilight hours and this corresponded to the lowest level of prey digestion (Fig. 3.11). Given the predominance of undigested prey, it can be assumed that the high feeding intensity stemmed from "recent" feeding activity relative to their time of capture. Though *M. walvisensis* did appear to feed a little more at night than at other periods (i.e. suggesting nocturnal activity), differences in feeding intensity and state of prey digestion were not significant for this species, with digested material dominating the stomach content irrespective of time (Fig. 3.11).

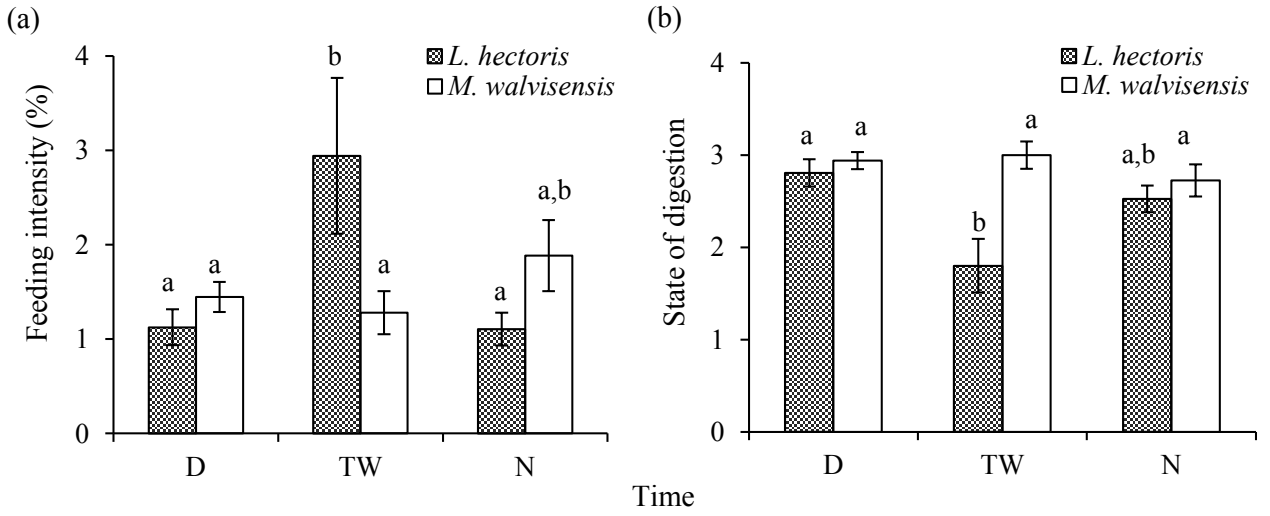


Fig. 3.11. The mean (a) feeding intensity (as percent wet body weight) and (b) state of digestion by time: daylight (D), twilight (TW), and night (N) for *Lampanyctodes hectoris* and *Maurolicus walvisensis*, pooled across both spring 2014 and autumn 2015 cruises**.** Letters represent the results of Tukey's *post-hoc* comparisons of group means, shared letters indicate values are not significantly different ($p > 0.05$).

3.3.6 Effect of fish size on feeding habits

The average length of ingested prey per individual fish was compared by species and season using a two-way ANOVA. Prey length differed significantly between the two mesopelagic species ($F_{(1,156)}$=7.207, p=0.0081), but no seasonal effect (p=0.38) or interaction were detected (p=0.95). *Lampanyctodes hectoris* consumed prey significantly larger in size (4.87±0.52mm) than *M. walvisensis* (2.86±0.53mm). Given that the upper limit of ingested prey is physically restricted by mouth gape, it is likely that the differences in ingested prey correspond to those seen in gape size between *L. hectoris* and *M. walvisensis* (refer to Fig. 3.2). Mean prey to gape size ratios were examined; *L. hectoris* and *M. walvisensis* ingested prey that were approximately 40% and 39% the length of their gape, respectively.

Of the two species studied, *L. hectoris* exhibited a significant positive relationship (p<0.001) between SL of individual fish and the average length of ingested prey (Fig. 3.12). Individuals belonging to *L. hectoris* fed on a range of prey sizes (as indicated by the spread of values), but the upper threshold of ingested prey was found to increase with increasing SL and therefore gape size. Although a size-related increase was observed for *M. walvisensis*, this relationship was strongly influenced by the presence of a group of outliers. Following their removal, no significant relationship between prey size and SL was detected. Nevertheless, the disparity observed in the (mean) prey size selected by *M. walvisensis* in this study suggests the presence of

two different feeding strategies utilized by this species (Fig. 3.12). The majority of *Maurolicus walvisensis* individuals fed indiscriminately on prey < 2.5mm length irrespective of fish size. However, a few larger (>35mm SL) individuals were found feeding on much larger prey (~15mm), indicating the potential to switch diets, namely from feeding on small meso-zooplankton (i.e. copepods) to larger macro-zooplankton (i.e. euphausiids). Individuals that fed on larger prey were sampled from several trawls across both seasons, suggesting that the observed diet switching may be a result of opportunistic foraging or possibly individual variation within the species.

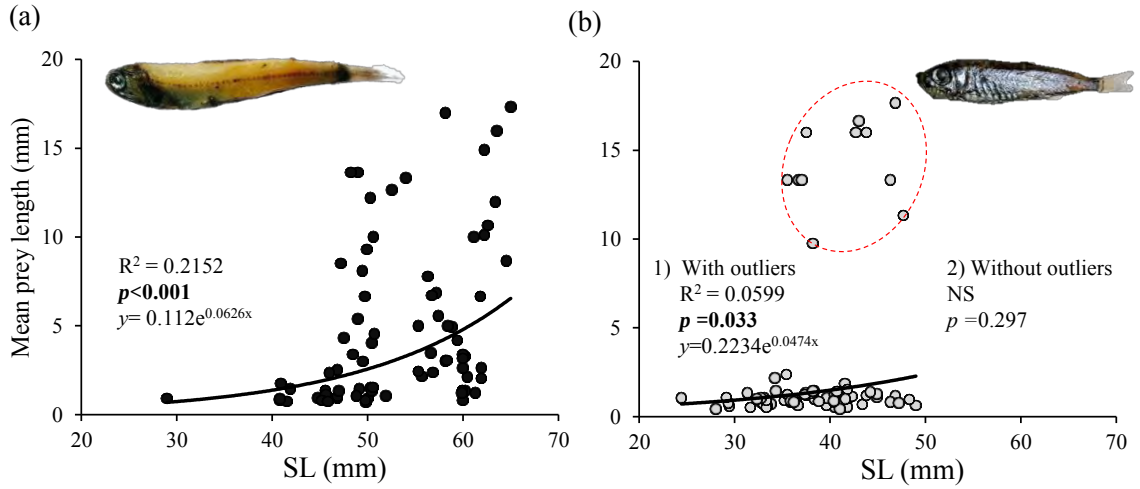


Fig. 3.12. Relationship between mean length of ingested and identifiable prey (±SE) and SL of individual fishes for (a) *Lampanyctodes hectoris* (n=77) and (b) *Maurolicus walvisensis* (n=79), with (1) and without (2) outliers (n=11) included. Exponential regressions, R^2 and *p*-values shown, significant values ($p<0.05$) are in bold. Non-significant (NS) relationships are indicated. Circle denotes outliers for *M. walvisensis* which suggests possible dietary switches within this species.

3.4 Trophic position estimates

Trophic positions calculated for *L. hectoris* and *M. walvisensis* depended on the method of analysis (SIA versus SCA) ($F_{(1,452)} = 9.767$, $p=0.0019$) and also on the reference organisms used to estimate the $\delta^{15}N$ baseline of the food web ($F_{(1,196)} = 823.9$, $p<0.001$). Of the two baseline estimates, the intertidal mussel *Mytilus galloprovincialis* resulted in the lowest estimate of trophic position for either species (Table 3.4). By contrast, applying the $\delta^{15}N$ of mixed phytoplankton from the northern Benguela as the baseline yielded the highest trophic estimates for *L. hectoris* and *M. walvisensis* (Table 3.4). These results indicate that literature derived baselines cannot be used with any certainty to estimate trophic position. Consequently, only trophic position estimates derived from dietary data were used for subsequent analyses.

Table 3.4. Mean trophic position, standard error (±SE) and range of trophic position values, and the number of samples for *Lampanyctodes hectoris* and *Maurolicus walvisensis,* pooled across spring 2014 and autumn 2015 cruises. Trophic positions were calculated using dietary and $\delta^{15}N$ methods. Two different estimates of $\delta^{15}N$ baseline, the intertidal mussel *Mytilus galloprovincialis* from the southern Benguela [65] and phytoplankton from the northern Benguela [64] were used. Letters represent the results of Tukey's *post-hoc* comparisons of group means; shared letters indicate values that are not statistically different (p>0.05).

			$\delta^{15}N$				
Species	$\delta^{15}N$ base	λ base	Mean trophic position	SE	Range	Tukey levels	*n*
L. hectoris	Mussel	2.4	3.93	0.01	3.65-4.16	a,f	50
M. walvisensis	Mussel	2.4	3.63	0.03	3.02-4.00	b	50
L. hectoris	Phytoplankton	1.0	4.64	0.01	4.35-4.87	c, e	50
M. walvisensis	Phytoplankton	1.0	4.34	0.03	3.73-4.70	d	50
			Dietary				
L. hectoris	---	---	4.21	0.03	3.50-4.50	e	77
M. walvisensis	---	---	3.85	0.03	3.32-4.50	f	79

Diet derived trophic positions varied significantly between the two species (ANOVA $F_{(1,152)} = 38.3$, $p<0.001$), with *L. hectoris* occupying a higher trophic position than *M. walvisensis* overall (Table 3.4). Seasonal differences within species were not significant ($p=0.57$), nor was the interaction between species and season ($p=0.63$). Though the fitted regressions have little to no predictive power as they explain less than 9% of the observed variability, the positive relationship between trophic position and standard length for both species were significant ($p<0.05$; Fig. 3.13). Furthermore, ANCOVA revealed that the rate of change in trophic position relative to SL was different between species ($F_{(1,252)} = 19.85$, $p<0.001$), and controlling for fish size, that trophic position significantly differed between species ($F_{(1,252)} = 8.162$, $p<0.001$). Consequently for fishes of equal size, *L. hectoris* occupied a higher trophic position than *M. walvisensis* (Fig. 3.13), consistent with the results reported for $\delta^{15}N$ values (see section 3.2).

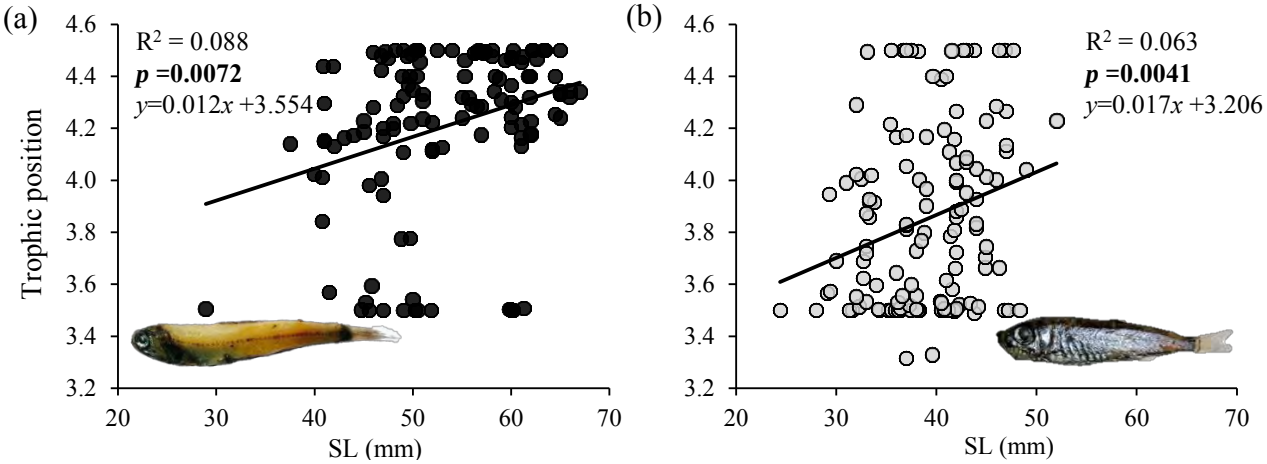


Fig. 3.13. Diet-derived trophic position vs. standard lengths (mm) for *Lampanyctodes hectoris* (n=77) and *Maurolicus walvisensis* (n=79), pooled across spring 2014 and autumn 2015 cruises. Linear regressions, R^2 and p-values shown, significant values ($p<0.05$) are in bold. Note that the relationships are of significance ($p<0.05$) despite the high variability (as indicated by low R^2 values).

Chapter 4: Discussion

This study investigated the feeding ecology of two abundant mesopelagic species, lanternfish *Lampanyctodes hectoris* and lightfish *Maurolicus walvisensis,* off the west coast of South Africa. To this end stable isotope and stomach content analyses were completed on fishes caught between Cape Point and Hondeklip Bay in spring 2014 and autumn 2015. The isotopic values of *L. hectoris* and *M. walvisensis* were examined for interspecific, seasonal, and size-related variability in their trophic levels ($\delta^{15}N$) and source production ($\delta^{13}C$). This study found that the two species occupied different isotopic niches, which differed by their $\delta^{15}N$ values. Though *L. hectoris* and *M. walvisensis* exhibited a similar $\delta^{13}C$ range, further analysis revealed that these two species differed significantly in their $\delta^{13}C$ central tendencies across both seasons in which they were sampled.

Both *L. hectoris* and *M. walvisensis* are zooplanktivorous consumers and though they showed some overlap in prey, the diets of these two species significantly differed in their overall composition. In terms of dietary carbon, this study found that *L. hectoris* fed predominantly on macro-zooplankton (i.e. euphausiids), while *M. walvisensis* fed mainly on meso-zooplankton (i.e. copepods). Nevertheless, the relative importance of various prey types varied to some extent by the time of year (Appendix D) and by the size of the predators. *Lampanyctodes hectoris*, the larger of the two species in terms of gape and standard length, fed on larger prey than *M. walvisensis* and the upper threshold of ingested prey was found to increase with increasing fish SL. By contrast, *M. walvisensis* appears to feed indiscriminately on small prey under a certain size threshold (~2.5mm) across its range of standard lengths; however, larger individuals may switch to larger prey (i.e. euphausiids) when conditions are favorable. Furthermore, feeding intensity for *L. hectoris* peaked during twilight, while *M. walvisensis* appeared to feed at lower levels throughout the day. However, these results which indicate asynchronous feeding periodicities should be confirmed with a dedicated sampling design and larger sample sizes.

4.1 The effect of lipid correction on mesopelagic stable isotope values

Stable isotope analyses are widely used in marine ecological studies, as $\delta^{15}N$ reflects the trophic position at which a consumer feeds [51], while $\delta^{13}C$ reflects the origin of organic matter

maintaining the food web [58]. However, $\delta^{13}C$ signals are strongly influenced by lipids present in the tissue of oily fish like these, with chemical lipid extraction being a favoured method used to remove lipid bias from bulk tissue $\delta^{13}C$ measurements [52,72]. As documented by Logan & Lutcavage [67] and de Lecea & de Charmoy [52], to name a few, this study also found that chloroform/methanol extractions were a suitable method to remove lipid content from the muscle tissue of mesopelagic fishes. However as expected from the literature, it also significantly altered the $\delta^{15}N$ signals in extracted samples.

These results confirm what other studies have suggested, namely that samples ought to be analyzed in duplicate to derive accurate $\delta^{13}C$ (lipid extracted) and $\delta^{15}N$ (non-treated) values [51,52,67]. While the source of $\delta^{15}N$ alteration is still not fully understood, the removal of amino acids attached to structural lipids by polar solvents has been proposed as a possible mechanism [72]. Nevertheless, given evidence for $\delta^{15}N$ alteration in fish muscle tissue, mathematical correction approaches should be further explored as an alternative to lipid extraction in oily mesopelagic fishes; thereby simplifying sample preparation, reducing analytical costs, and better preserving the integrity of samples for $\delta^{15}N$ analysis [71].

4.2 Stable isotope ratios of mesopelagic fishes

Using nitrogen stable isotope ratios ($\delta^{15}N$) as an indicator of trophic position [51], this study determined that *L. hectoris* sampled in both the spring and autumn cruises consistently occupied a higher trophic position relative to *M. walvisensis* from the Cape Point – Hondeklip Bay region. Larger zooplankton taxa, like euphausiids and amphipods, are more enriched in their $\delta^{15}N$ values than smaller meso-zooplankton [7,8,33,94]. With that in mind, the two different species in isospace (separated mainly by their $\delta^{15}N$ range) suggest different feeding strategies, with the larger species (*L. hectoris*) foraging on larger prey and the smaller species (*M. walvisensis*) on smaller zooplankton. These patterns were supported by the observed differences in diet composition. However, they contrast with those of Davenport & Bax [95], who compared the $\delta^{15}N$ values of *L. hectoris* (10.2‰; n=10) and *M. muelleri* (10.6‰; n=5), a closely related congener of *M. walvisensis*, off the continental slope of southeastern Australia, and detected no significant difference in their relative trophic positions. The discrepancy may be an artefact of sampling size, however, as data collected by Davenport & Bax [95] used to examine broad scale tropho-

dynamics of the shelf ecosystem were potentially inadequate to detect fine scale differences between the two species in question.

When compared with other ecosystems, the mean $\delta^{15}N$ signal for *M. walvisensis* was markedly higher than values reported for *M. muelleri* or other sternoptychids in the Mediterranean [94], northeast Atlantic [96], Gulf of Mexico [33] and off south-eastern Australia [95]. Similarly, $\delta^{15}N$ of *L. hectoris* was also higher than values reported for conspecifics sampled in south-east Australia [95], as well as for myctophid fishes studied elsewhere [7,94,96]. Assuming that methodological differences are minimal (as these studies either mathematically corrected for lipid content or chemically extracted in duplicate, thereby preserving the integrity of $\delta^{15}N$), these discrepancies likely stem from different $\delta^{15}N$ values characterizing the base of these food webs, which are often ecosystem-specific and prone to spatio-temporal variation [57].

Although standardizing $\delta^{15}N$ values against an isotopic "baseline" [54,62] provides a continuous measure of an organism's trophic position amenable to comparative multisystem studies of trophic structure [97], the accuracy and appropriate selection of a $\delta^{15}N$ baseline is essential [57]. When baseline estimates poorly represent the food web base and/or represent a single point of a continuum which otherwise varies over time, trophic predictions can falter [57, 61]. This study examined the use of literature-derived baselines (from the Benguela region) and found that the trophic positions calculated for *L. hectoris* and *M. walvisensis* varied extensively by the type of baseline applied (littoral mussel vs. phytoplankton). In response, these baselines could not be used with any certainty to estimate the trophic position of mesopelagic fishes for cross-system comparisons. In line with reviews which call for more careful characterization of isotopic baselines in ecological studies [57,60,61]; the results of this study suggest that literature-derived baselines should be used with caution and avoided when possible.

Using carbon stable isotope ratios ($\delta^{13}C$) to infer the origin of organic matter maintaining the food web [51], *L. hectoris* and *M. walvisensis* exhibited a similar $\delta^{13}C$ range (~2.5‰) in isospace. Since the $\delta^{13}C$ range gives an estimate of the diversity of basal sources, the narrow range suggests that the pelagic food web between Cape Point and Hondeklip Bay is likely supported by only a few sources of primary production. Though there is a strong seasonality within the phytoplankton community of the southern Benguela (i.e. large-celled diatoms and dinoflagellates typi-

cally dominate during the summer and smaller-celled dinoflagellates are more dominant in winter) [98], these results are consistent with the few but abundant primary producers which typify the region.

Despite the similar $\delta^{13}C$ range within the mesopelagic assemblage, *M. walvisensis* and *L. hectoris* were nevertheless found to differ in their central tendencies for $\delta^{13}C$ across both the spring and autumn surveys. Though these differences may stem from species-specific and/or size-related differences in the assimilation of dietary carbon [99,100], they may also result from *M. walvisensis* and *L. hectoris* using somewhat different foraging habitats. Within a biogeographical context, different $\delta^{13}C$ gradients exist in the marine environment that can be used to elucidate the feeding behaviour and foraging habitat of consumers [7,58]. Differences in $\delta^{13}C$ values can indicate inshore versus offshore feeding [101], pelagic versus benthic contribution to food intake [102], and/or latitudinal changes in foraging habitat (Fig. 4.1) [7]. Although this study could not separate spatial factors (like depth, latitude, and longitude) due to limited sampling, other studies have observed mesopelagic assemblages segregating by the depth at which species feed or by their proximate position to the shelf edge [3,7,103]. Such differences in habitat utilization may reduce inter-specific competition in the mesopelagic assemblage and facilitate the competitive coexistence of fishes that otherwise feed on a common resource [7].

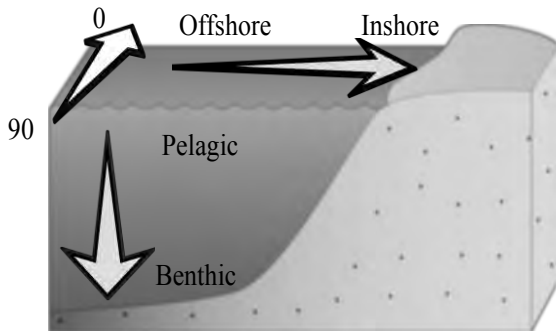


Fig. 4.1. The 3-dimensional gradient in $\delta^{13}C$ enrichment observed in the marine environment; arrows indicate the direction of increasing $\delta^{13}C$ with increasing depth, and with proximity to the equator and the coast, see text for details.

Of the two mesopelagic fishes, *L. hectoris* exhibited notable size-related changes in isotopic values and this was largely attributed to the greater range of fish lengths sampled for this species. However, observed size-related shifts are not nearly as prominent as those observed within species which exhibit true ontogenetic (i.e. developmental) shifts in diet, like the deep and shallow water hakes *Merluccius* spp., which shift from facultative zooplanktivory to piscivory with size [104]. Nevertheless, the increase in $\delta^{15}N$ with increasing size has been documented for other myctophid species [7, 33, 94], and likely indicates an increasing importance of euphausiids (or fish) and decreasing importance of copepods and other small zooplankton as individuals

grow. The absence of size-related shifts in isotopic values for *M. walvisensis* suggests opportunistic predation across their SL-range (i.e. high feeding plasticity) and hence successful feeding in highly variable environments. Although the $\delta^{15}N$ values reported here can be used to estimate the relative trophic position of difference size classes within the mesopelagic assemblage, it should be done with caution [104], as the variation in trophic fractionation of consumers can be substantial within a species (that is among individuals of different sizes, etc.) [51].

4.3 Diet composition of mesopelagic fishes

Combining stable isotope and stomach content analyses remains a fundamental approach to clarify trophic relationships, because two or more food sources can possess identical isotopic signatures, which introduces uncertainties in the interpretation of isotopic data [62]. *Lampanyctodes hectoris* and *M. walvisensis* sampled between Cape Point and Hondeklip Bay were broad consumers of meso- and macro-zooplankton and prey included crustaceans (euphausiids, amphipods, copepods), ichtyoplankton, and mollusc larvae. Although occasional herbivory has been documented in mesopelagic fishes elsewhere [3,105], phytoplankton was absent from stomachs examined. In addition, gelatinous zooplankton (i.e. salps, tunicates, and chaetognaths) are relatively abundant off the west coast of South Africa [6,106], yet they were similarly absent from the stomach content of *L. hectoris* and *M. walvisensis*. Their absence may be attributed to two factors: (1) the differential digestion of soft bodied prey [73] and/or (2) reduced predation associated with the limited visibility of translucent-opaque prey [32]. With that said, however, gelatinous taxa have been reported in the diets of mesopelagic fishes studied elsewhere, though their dietary importance is usually negligible [13,32].

Lampanyctodes hectoris is the only species in this study for which food habits have been described in the peer-reviewed literature. On the whole, the diet composition observed in this study was consistent with those of earlier reports for *L. hectoris* off the west coast of South Africa [29] and in the shelf waters of Tasmania [13,35]. More specifically, Prosch [29] found that copepods, amphipods, and euphausiids were the main prey items taken, reported here in descending order of (presumed) numerical frequency. Young & Blaber [13] found that *L. hectoris* off Tasmania fed predominantly on euphausiids and secondarily on calanoid copepods; however the contribution of amphipods was negligible. Tyler & Perry [31] found that the diets of three

myctophid species off the Oregon coast were heavily dominated by the euphausiid *Euphausia pacifica* and medium to large copepods, of which the most frequently identified belonged to *Calanus* sp. and *Metridia* sp. Gjosaeter [107] showed similar results for another high latitude myctophid, *Benthosema glaciale*, however *Thysanoessa* spp. were the dominant euphausiids consumed. The results of this study are in agreement with the general literature, in that myctophid fish are largely macro-zooplanktivores and feed extensively on euphausiids, though meso-zooplankton prey are often next in importance.

While the diet of *M. walvisensis* has not been described in the peer-review literature, the description presented in this study was similar to that of *M. muelleri*, a closely related congener with circumpolar distribution. Carmo *et al.* [17] found that *M. muelleri* sampled over the northern mid-Atlantic ridge fed predominantly on calanoid copepods and euphausiids, which are prey items frequently reported for this species. However, cladocerans were also important for larger individuals [17] and were similarly described as a food source for *M. muelleri* by Ikeda *et al.* [14] in the southern Japan Sea, and by Rasmussen and Giske [108] in the northeast Atlantic during the summer period. Though cladocerans are found in the southern Benguela to an extent, their overall abundance is low relative to other zooplankton taxa (i.e. copepods)[106] and may be more prevalent in the south coast sub-system [84], thereby providing a plausible explanation for their apparent absence in the diet of *M. walvisensis* sampled from the west coast in this study. In the Canary Current upwelling system off northwestern Africa, Samyshev & Schetinkin [16] showed similar findings as those presented in this study. The diet of *M. muelleri* was heavily dominated by euphausiids, crustacean larvae, and calanoid copepods, of which the most frequently identified belonged to *Calanus* sp. and *Candacia* sp. [16]. Overall, this study is consistent with the general literature, confirming that the sternoptychid *Maurolicus* spp. is predominantly meso-zooplanktivorous, with calanoid copepods contributing significantly to its diet.

4.4 Resource partitioning within the mesopelagic assemblage

Studies have shown that aspects of alimentary morphology (mouth structure, gill rakers, branchial apparatus, etc.), more often than not, reflect the feeding behaviours of fishes, particularly among those that ingest prey whole (i.e. suction feeders) [92,109,110]. The notion that gape size is an important constraint on prey type is widespread in eco-morphological studies, and is

often cited as a possible explanation for correlations seen between prey type and predator size [111]. Yet, relatively few studies measure either gape size or indices of gape (i.e. length of jaw) and its effects on diet composition [111]. Consequently, one of the objectives for this study was to investigate resource partitioning between *L. hectoris* and *M. walvisensis* sampled between Cape-Point and Hondeklip Bay in the spring 2014 and autumn 2015 surveys.

It is reasoned that a fish's gape width determines its ability to trap its prey, while its gape length (i.e. jaw length) determines the size of its prey [92,111]. *Lampanyctodes hectoris* is characterized by a terminal mouth with large, hinged jaws that are lined with rows of small teeth (Fig. 4.2a) [24]. Such mouths are usually associated with predatory midwater fishes that are often capable of hyper-extending their jaws to ingest large prey [109, 112].By contrast, *M. walvisensis* is characterized by a superior (ventrally oriented) mouth typically associated with surface feeders (Fig. 4.2b) [110]. However, relative to other teleosts, the upper jaw is shortened to limit the gape, yet maintains the expansibility of the buccal cavity, which thereby increases net suction force [109].

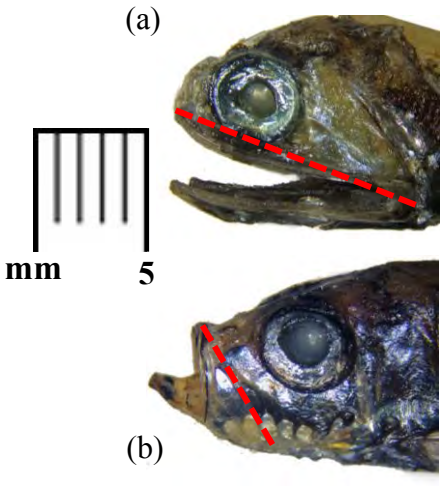


Fig. 4.2. The mouth structure and gape size for (a) *Lampanyctodes hectoris* and (b) *Maurolicus walvisensis* of equal standard lengths (40.5mm SL). Upper jaw length (i.e. the maxilla and premaxilla) used as an indicator of gape is shown by the dashed lines.

While the two species showed some overlap in the range of standard lengths sampled (≃40-50mm SL), *L. hectoris* possessed a larger gape (as indicated by jaw length) than *M. walvisensis* of similar size and the difference was largely attributed to their respective mouth structures (Fig. 4.2). *Lampanyctodes hectoris* should therefore eat larger prey than *M. walvisensis* of equivalent SL, as substantiated by the feeding habits reported in this study. Despite feeding predominantly on small prey, the results indicate that *M. walvisensis,* particularly larger individuals, can switch diets (i.e. from meso-zooplanktivory to macro-zooplanktivory) under certain conditions (Fig. 3.12). Dietary switches have been previously documented in the literature for other mesopelagic fishes [9,35] and though the cause is mostly unknown, these observations are thought to be linked to changes in prey availability, the absence of competitor species, and/or opportunistic foraging [110].

The food base is in constant dynamic change (i.e. with respect to its total biomass and species composition) and such stochasticity can be accommodated by diet switches, which reflects a level of trophic adaptability on behalf of the consumer [110]. One proposed mechanism as to why a consumer may switch from feeding on one resource to another is that it may increase an individual's foraging efficiency and therefore its overall fitness with time [110]. In this instance, a single euphausiid (measured in this study) was comparably more nutritious in terms of its dietary carbon (≃2,000μg) than a single copepod (≃20μg), and it therefore represents an energy bonus for individuals that successfully predate on larger prey. Nevertheless, the ability for *M. walvisensis* to switch between feeding strategies, in conjunction with its broad dietary breadth, makes this species a highly opportunistic and flexible forager in a dynamic and unpredictable environment [3,107]. By contrast, *L. hectoris* possessed a narrower dietary breadth and isotopic niche (in terms of its $\delta^{15}N$ range), which suggests some degree of dietary specialization on macro-zooplankton. Nevertheless, prey cumulative curves indicate that sampling may have been insufficient to fully describe the dietary habits of either species, particularly *M. walvisensis*, highlighting the need for larger sample sizes in future studies.

In addition to partitioning the zooplankton resource on the basis of size, differences in feeding intensity and prey digestion state between *L. hectoris* and *M. walvisensis* suggest the possibility of asynchronous foraging activity. Acoustic surveys in the southern Benguela documented that *L. hectoris* undergoes extensive vertical migrations, ascending at dusk and descending at dawn [1]. Although sampling was limited, the preliminary results from this study were consistent with their diel cycle and suggest a tight coupling between vertical movement and feeding activity for *L. hectoris*. By contrast, no feeding periodicity was detected for *M. walvisensis*, with well-digested material dominating the stomach content irrespective of time. Though *M. walvisensis* is known to undertake significant vertical migrations in the Benguela region [11], these results suggest that either feeding activity occurs throughout the day, digestion rates are slow, or that sample sizes were inadequate. Documentation is lacking for *M. walvisensis* and earlier reports of the feeding periodicity of *M. muelleri* are inconsistent; both nighttime feeding [35,108] and no diel feeding rhythm [16,107] are reported. Dedicated sampling throughout the diel cycle of either species would therefore be necessary to improve our understanding of their feeding periodicities.

4.5 Seasonal variability in the feeding habits of mesopelagic fishes

Complex spatial and temporal variations in prey size and biomass typify pelagic food environments [18,113]. Upwelling systems like the southern Benguela are further characterized by intense and persistent pulses of upwelling in spring to late summer and very weak to absent upwelling during the late autumn to winter [18]. In that regard, studies have documented that the abundance of zooplankton (particularly euphausiids and large copepods) increases in the spring, corresponding to the peak in production, followed by a decline in the autumn [18,113]. However, despite the purported fluctuations in zooplankton abundance, the lack of seasonal changes in the diet composition of *L. hectoris* and *M. walvisensis* analysed in this study suggests that food was not limiting between Cape Point and Hondeklip Bay for mesopelagic fishes during the 2014 – 2015 study period.

Stable isotope analysis examines dietary information assimilated over weeks to months (depending on the turnover rate of the tissues examined) [51], while stomach content analysis represents but a "snap-shot" of an organism's diet that varies over time [73]. Although seasonal variability was absent within the diet composition of *M. walvisensis,* this species did exhibit significant seasonality in its isotopic composition. Thus, stable isotope analyses may be more sensitive to dietary changes which otherwise go undetected using conventional techniques (i.e. stomach content analyses) [54]. *Maurolicus walvisensis* sampled in the autumn survey were more enriched in both $\delta^{15}N$ and $\delta^{13}C$, but were also significantly larger in size than spring conspecifics. Consequently, the observed difference in isotopic composition of this species from spring to autumn could be attributed to (1) the increase in fish size, (2) the increased abundance of larger zooplankton, or (3) possibly some combination of the two.

By contrast, *L. hectoris* did not vary in its isotopic niches or central tendencies between the spring and autumn surveys; nor were there differences in the standard lengths of fish sampled. These results further support the notion that *L. hectoris* is a selective predator that preferentially exploits macro-zooplankton. Conversely, the seasonal variability in the isotopic composition for *M. walvisensis* (coupled with its broad dietary breadth and capacity to switch diets) further supports the supposition of it being an opportunistic forager. Nevertheless, both species

showed seasonal variation in their lipid content (C:N ratios) and this was found to correspond to seasonal patterns in their reproductive activity [4,29], for more details see Appendix C.

4.6 Trophic position estimates of mesopelagic fishes using isotopic and dietary data

The concept of trophic structure rests on a system of step-wise trophic levels, each of which embraces those organisms that feed on similar food sources [110]. Photosynthetic organisms form the base of any aquatic system and a variety of primary, secondary, and tertiary consumers make up higher trophic levels [114]. A single species may, however, feed at more than one trophic level [54,97], highlighting the need for both stomach content and stable isotope analyses to elucidate the trophodynamics of an ecosystem. As zooplanktivorous predators, mesopelagic fishes are purported to occupy the tertiary level of the pelagic system worldwide [3,7,20]. However, the range of trophic positions derived from dietary data for *L. hectoris* and *M. walvisensis* populations sampled between Cape Point and Hondeklip Bay encompassed one full trophic position, which suggests that these fishes exhibit some degree of dietary plasticity, and quite possibly, trophic adaptability. Nevertheless, these results confirm what other studies have suggested [7,33,94,115], namely that sternoptychid and myctophid fishes represent not only secondary but tertiary consumers in the pelagic environment.

In the southern Benguela, a number of studies have investigated the trophic links among pelagic nekton common to the region using ecosystem models [10,20] and stable isotope analyses ($\delta^{15}N$) [104,116], the results of which are summarized in Fig. 4.3. Mass-balanced trophic models in particular have been used to investigate food web structure and function in marine systems [20] and the reported values are typically considered to be sound estimates of trophic position that can be used for explicit comparisons with those inferred from stomach content observations. Both Shannon *et al.*[20] and Osman [10] estimated the trophic position of mesopelagic fishes (treated as a single functional unit) to be in the order of 3.6 – a value derived using initial state input data from the mid-1980s. Although the trophic position of *M. walvisensis* in this study closely matched that of the model-derived estimate for the functional group, *L. hectoris* was shown to feed at a higher position than predicted by ecological models (Fig. 4.3). While trophic positions calculated in this study are likely robust for the period (2014-2015) and region sampled (Cape Point -Hondeklip Bay), further sampling is needed to validate these observations through-

out the southern Benguela and across longer time scales before well-founded recommendations can be given to improve the way mesopelagic fishes are modelled in the region.

In the absence of adequate and reliable baseline data for primary producers and/or consumers (section 4.2), $\delta^{15}N$ of *L. hectoris* and *M. walvisensis* were compared against the $\delta^{15}N$ values of other pelagic species in order to infer relative trophic positions within the southern Benguela (Fig. 4.3). Within the context of these cross-study comparisons, mesopelagic fishes sampled in this study appear to feed at a higher trophic position than sardine, as suggested by both diet and isotopic data (Fig. 4.3). This observation is consistent with the differences in their known feeding habits. Sardine possess a relatively fine branchial apparatus and feed predominantly by filter-feeding on phytoplankton, but derive the bulk of their dietary carbon from meso-zooplankton (i.e. calandoid and cyclopoid copepods) [84]. The trophic positions of anchovy, herring, and juvenile horse mackerel are consistent with that of *M. walvisensis*, all of which are facultative meso-zooplanktivores that derive the bulk of their dietary carbon from meso-zooplankton, but can feed on larger prey like euphausiids [6,38,39]. By contrast, *L. hectoris* appeared to feed at a position more consistent with large horse mackerel, squid, and small deep- and shallow-water hakes (based on $\delta^{15}N$ data; Fig 4.2). These species are all facultative zooplanktivores that feed extensively on large macro-zooplankton like euphausiids, but are also known to complement their diets with fish [6,117]. Nevertheless, in agreement with the purported stepwise $\delta^{15}N$-enrichment between prey and predators [51], the trophic positions of *L. hectoris* and *M. walvisensis* were significantly lower than those of their well-known predators, such as snoek, and large shallow-water and deep-water hakes (Fig. 4.2) [6,11].

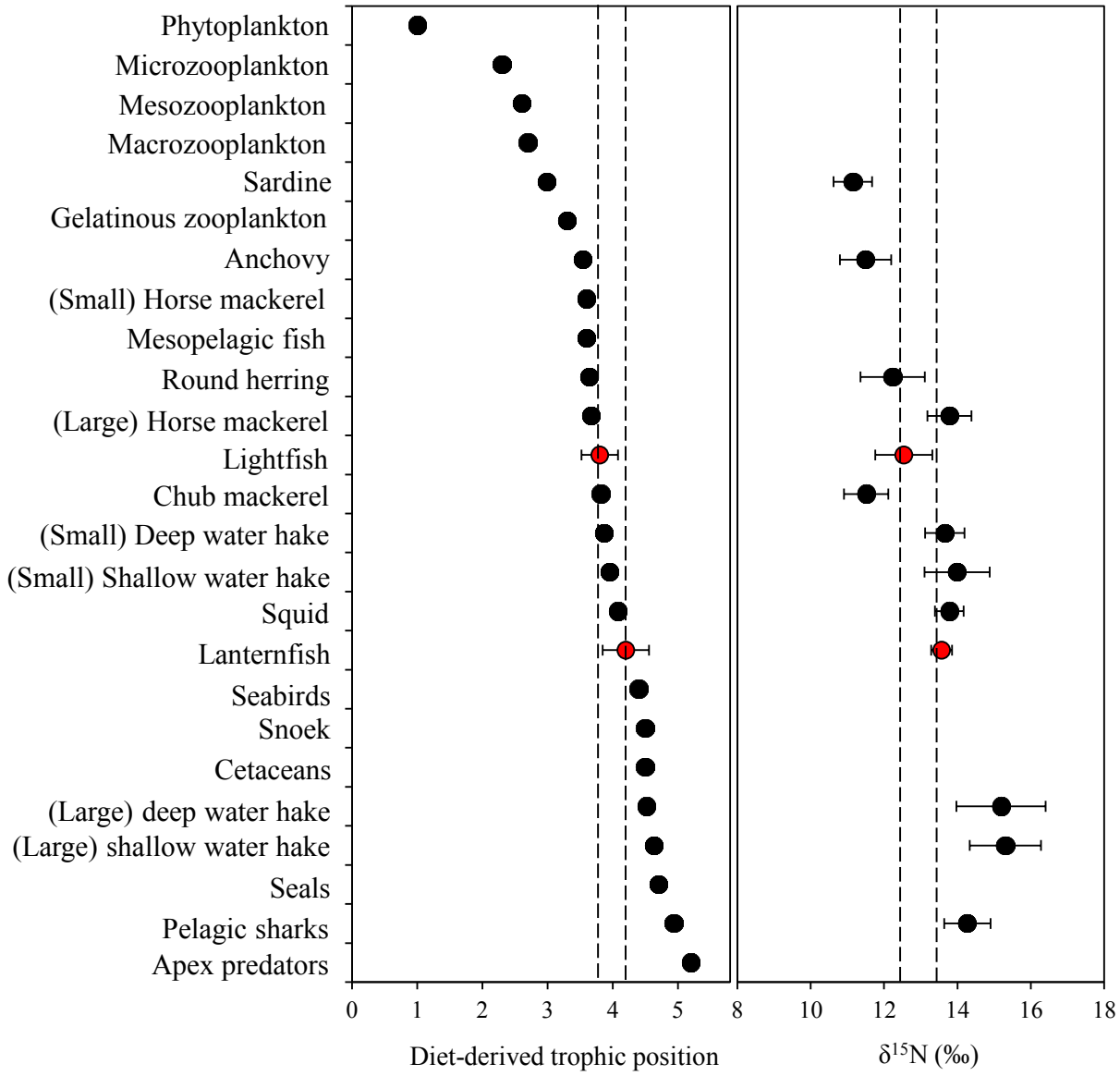


Fig. 4.3. Dietary and isotopic ($\delta^{15}N$) data used to infer trophic positions of planktonic and nektonic organisms from the southern Benguela, here ranked by model-derived trophic positions. Dashed lines represent the mean trophic position of *Lampanyctodes hectoris* and *Maurolicus walvisensis* respectively, and standard deviations are shown. Model-derived trophic positions were extracted from Osman [10] and isotopic values from van der Lingen & Miller [116].

Chapter 5: Conclusion

This study set out to examine the feeding ecology of two mesopelagic fishes, the lanternfish *Lamanyctodes hectoris* and the lightfish *Maurolicus walvisensis*, in the west coast sub system of the southern Benguela through stomach content and stable isotope analyses. Data was collected in order to achieve the following objectives:

- Stable isotope signals of *L. hectoris* and *M. walvisensis*, in particular $\delta^{15}N$, were compared for trophic segregation within the assemblage.
- The diet of *L. hectoris* and *M. walvisensis* were quantified in terms of the frequency of occurrence, numerical abundance, and contribution to dietary carbon derived from stomach contents.
- Seasonal variability was investigated in the diet and/or isotopic composition of both species and size related artefacts possibly contributing to the observed seasonal patterns were similarly examined.
- The methodological application of stable isotope analysis was examined, namely the effect of chemical lipid extraction on isotopic values of mesopelagic fishes and the effect of using literature derived isotopic baselines to standardize $\delta^{15}N$ values for cross-study comparisons.
- Resource partitioning between *L. hectoris* and *M. walvisensis* was examined, here achieved by comparing the mean prey lengths ingested, overall dietary diversity, and feeding periodicity of each species, in order to infer their respective feeding strategies.
- Size-related shifts in isotopic ratios, mean prey lengths ingested, and trophic positions for both *L. hectoris* and *M. walvisensis* were investigated.
- Trophic position estimates for *L. hectoris* and *M. walvisensis* obtained in this study were compared in relation to those derived from ecological models and from stable isotope analyses available in the southern Benguela.

Through dietary and stable isotope analyses of the lanternfish *Lampanyctodes hectoris* and lightfish *Maurolicus walvisensis* sampled during the spring 2014 and autumn 2015 pelagic surveys between Cape Point and Hondeklip Bay, the results indicate that:

- Lipid extraction was necessary for these two mesopelagic fishes, as indicated by their high C:N ratios, and analyzing samples in duplicate was necessary to derived accurate $\delta^{13}C$ (lipid-extracted) and $\delta^{15}N$ (non-extracted) values.
- The two species occupied largely different isotopic niches separated by their $\delta^{15}N$ values, suggesting trophic segregation within the mesopelagic assemblage, with the larger species (*L. hectoris*) feeding higher up in the food web than the smaller species (*M. walvisensis*).
- Both *L. hectoris* and *M. walvisensis* are zooplanktivorous consumers and though they showed some overlap in prey, the diets of these two species significantly differed in

their overall composition, with *L. hectoris* deriving the bulk of its dietary carbon from larger macro-zooplankton (euphausiids) and *M. walvisensis* from meso-zooplankton (copepods).

- Some resource partitioning between *L. hectoris* and *M. walvisensis* observed in this study was likely facilitated by differences in their alimentary morphology and foraging strategies (i.e. opportunistic vs. specialized predation), and possibly by asynchronous feeding activity.

- *Lampanyctodes hectoris*, the larger of the two species in terms of gape and standard length, fed on larger prey than *M. walvisensis* and the size of ingested prey increased with increasing fish SL. *Maurolicus walvisensis* appeared to feed indiscriminately on small prey under a certain length (~2.5mm) across its range of standard lengths. However, some individuals had the capacity to switch to larger prey, which suggests diet switching when conditions are favorable.

- Both species exhibited size related shifts in trophic position with increasing SL, which suggests that intraspecific competition may be mediated by resource partitioning and associated adaptive features. *Lampanyctodes hectoris* was found to consistently occupy a higher trophic position than *M. walvisensis*, and both species could be classified as secondary and tertiary consumers in the open ocean (in the context of this study).

Inadequate sampling over years, seasons, and areas has restricted the ability of this study to fully describe the feeding ecology of mesopelagic fishes in the southern Benguela. Consequently, further research would be needed to:

- Explore mathematical models as an alternative to lipid extraction for correcting lipid bias in oily mesopelagic fishes, as it would simplify sample preparation, reduce analytical costs, and better preserve the integrity of samples for $\delta^{15}N$ analysis.
- Elucidate long term seasonal and inter-annual patterns in the isotopic values (i.e. trophic positions) for both mesopelagic fishes. Stable isotope analysis complemented rather than replaced stomach content analysis, but it appeared to be more sensitive to seasonal differences than conventional techniques. However, further analysis is required to separate purported seasonal differences from potential size effects.
- Validate the diet composition of *L. hectoris* and *M. walvisensis* over longer time periods and throughout the southern Benguela region, as well as to examine prey preference and diet switching with respect to the abundance and composition of zooplankton available in the ambient environment.
- Better understand the feeding periodicities of *L. hectoris* and *M. walvisensis*, which would require dedicated sampling throughout the diel cycle of each species and also at various depths with time.
- Validate the trophic position estimates for *L. hectoris* and *M. walvisensis* (and possibly other mesopelagic species omitted in this study) throughout the southern Benguela and across longer time scales to derive robust trophic positions for these species and to form well-founded recommendations for improving the way mesopelagic fishes are modelled in the region.

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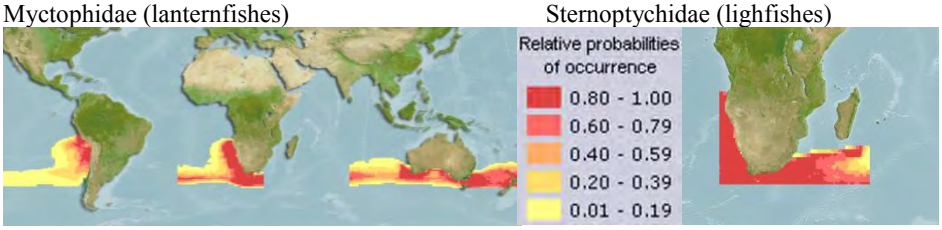
Appendix A Meristic and morphometric comparisons

Table A.1. A comparison of the meristic, morphometric, and life history traits of *Lampanyctodes hectoris* and *Maurolicus walvisensis,* as well as a summary of their commercial exploitation in southern Africa.

Species	*Lampanyctodes hectoris*	*Maurolicus walvisensis*
Family	Myctophidae (lanternfishes)	Sternoptychidae (lighfishes)
Distribution [d]		
Depth range	-----	271 - 1524 m (usually 300 - 400 m) [i, j]
Meristic & morphometric traits		
		
Mouth position	Terminal [a]	Superior
Standard Length	Max 73mm [k]	Max 47 mm [i]
Gill rakers	28-33, enlarged blade-like [g]	25-29 (usually 26-27) [i]
Vertebrae	----	33-35 [i]
Photophores	Present, number not described [a, k]	Present, 22-27 (usually 24-26) [i]
Swim-bladder	well developed, gas-filled [a, k, j]	well developed, gas-filled [i, j]
Life history traits		
Life span	1-3 years [k]	1-5 year [i, j]
Length/ age at sexual maturity	50 mm SL and age of one year [j]	-----
Spawning season	winter to spring season, peaking July and August [h, j]	winter to spring season (July-Nov) [h, j]
Fecundity	572 to 1431 eggs·g^{-1} [j]	161 to 738 eggs·g^{-1} [j]
Biochemistry		
Lipid content (% wet weight)	9.3 to 31.2% [e, h, j]	3 to 19.3% [e, h, j]
Wax esters (% lipid content)	10-90% [e]	15.30% [e]
Protein content (% wet weight)	14-15% [j]	13.5-18.9% [j]
Caloric value	23.2-29.8 kJ/g [j]	22.5-23.2 kJ/g [j]
Fisheries		
SA estimated stock	710,000 – 1,250,000 tonnes [b, f]	750,000 tonnes [b, f]
Commercial fishery	Limited commercial fishery, 100 – 42,000 tonnes (historically) [c, l]	Not taken commercially, occasionally recorded in mixed catches [c, l]
Experimental fishery	Mid-water (pelagic) trawl fishery as of 2011; catches from 2011-2012 totalled 9,486.5 tonnes, 83% consisted of lanternfish and 4% of lightfish [l]	
Management	An annual precautionary upper catch limit of 50,000 tonnes has been set for mesopelagic fishes from 2012 onwards [c, l]	

[a] Catul [24]
[b] Coetzee [1]
[c] DAFF [45]
[d] Froese & Pauly (FishBase) [118]
[e] Gjøsaeter & Kawaguchi [3]
[f] Hulley [2]
[g] Hulley [27]
[h] Hulley & Prosch [4]
[i] Parin & Kobyliansky [26]
[j] Prosch [11]
[k] Young *et al*. [119]
[l] van der Lingen [120]

Appendix B Alternate resources & the mesopelagic fishery

At present, most of the conventional fisheries are either fully or over-exploited worldwide, resulting in an urgent need to investigate alternate resources, including those of mesopelagic origin [3,121]. Although mesopelagic fisheries have a huge catch potential based on the stock biomass alone, there are only a few examples of mesopelagic fisheries in the world [3]. At present commercial fisheries include limited operations off South Africa, in the Southern Ocean, and the Gulf of Oman [24]. South Africa's fishery for mesopelagic fishes was established following the collapse of sardine and Cape horse mackerel stocks in the early 1960s, where purse seines with smaller mesh sizes (11-12.7 mm mesh) were introduced to catch anchovy for a reduction fishery [122]. However, the reduced mesh size additionally allowed for the capture of *L. hectoris*.

While under limited commercial exploitation in the southern Benguela, the mesopelagic catch has historically fluctuated between 100 and 42,400 tonnes and has accounted for some 10% of the total annual catch made by South Africa's small pelagic fishery in some years (Fig. A.1) [45]. However, the fishery intermittently closed during mid-80s due to processing difficulties caused by the high wax ester content of the fish [4]. Consequently, *L. hectoris* is now processed with other less oily fish to prevent the malfunction of existing reduction facilities [29]. In addition, the high content of wax esters renders it unsuitable for human consumption, so the catch serves as a replacement for pilchard in the production of fish oil and fish meal [11]. *M. walvisensis* is not taken commercially, but small quantities of *M. walvisensis* are recorded in mixed catches [45] and a large population is purported to exist on the west coast [1].

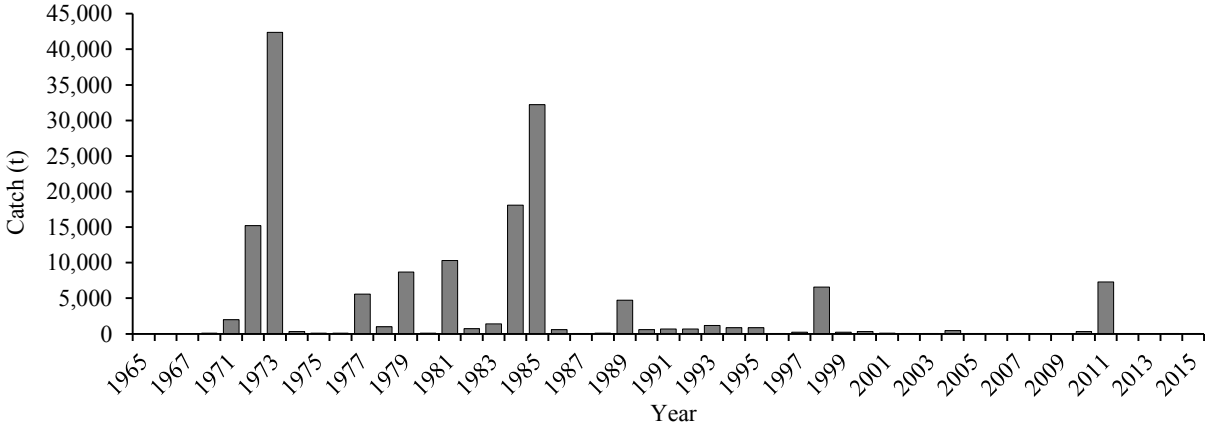


Fig. B.1. The catch in tonnes for mesopelagic fishes taken by the commercial and experimental fisheries off the west coast of South Africa (1965-2015). Although not species-specific, catches were heavily dominated by a single species *Lampanyctodes hectoris*. Catch data provided by DAFF (Branch: Fisheries Management).

In addition to the commercial purse-seine fishery, DAFF granted two-year permits in 2010 for an experimental mid-water trawl fishery targeting mesopelagic and pelagic stocks. Of the total catch reported for both years combined (9,486.5 tonnes), 83% consisted of *L. hectoris* and 4% of *M. walvisensis* [120]. In response, an annual precautionary upper catch limit of 50,000 tonnes has been set for mesopelagic fishes from 2012 onwards in order to sustainably manage catches of mesopelagic stocks [45]. Nevertheless, the possible effects of removing a sizable fraction of myctophids from the southern Benguela by commercial operations has yet to be examined and raises the issue of the effect on their zooplankton prey, competitor species, and predators.

Appendix C Inter-specific & seasonal varia

Two-way ANOVA revealed that non
C:N ratios differed significantly between the
fish *Lampanyctodes hectoris* and lightfish *Mau*
walvisensis ($F_{(1,152)}$ =7.11, p <0.001). More
cally, *post-hoc* tests indicate that *L. hectoris* h
nificantly higher C:N ratios than *M. walvisensi*
autumn (p<0.001), but not in the spring (p
Fig. C.1). C:N ratios also varied by season f
species ($F_{(1,152)}$ =183.911, p<0.001); fishes s
in the autumn had significantly more lipid
than their spring conspecifics (Fig. C1).

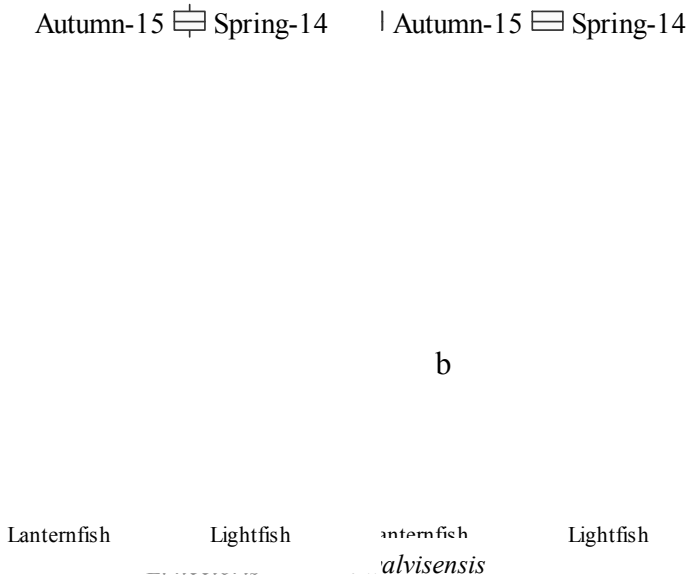


Fig. C.1. Boxplots of non-extracted C:N ratios by season, spring 2014 and autumn 2015, and by species, *Lampanyctodes hectoris* (spring n=25, autumn n=25) and *Maurolicus walvisensis* (spring n=25, autumn n=25). Letters represent the results of *post-hoc* comparisons of group means; shared letters indicate values that are not significantly different (p > 0.05). The median, interquartile range, min and max values (whiskers), and (•) outliers, as determined by R software, are shown.

Relationships between SL and C:N ratios were also investigated for *L. hectoris* (Fig. C.2) and *M. walvisensis* (Fig. C.3), separated by season. Although no size related shifts were detected during the spring cruise for either species, fishes sampled in the autumn exhibited increased lipid content with increasing SL. Only in the case of *M. walvisensis*, however, was the positive relationship of significance (p=0.0052; Fig. C.3b).

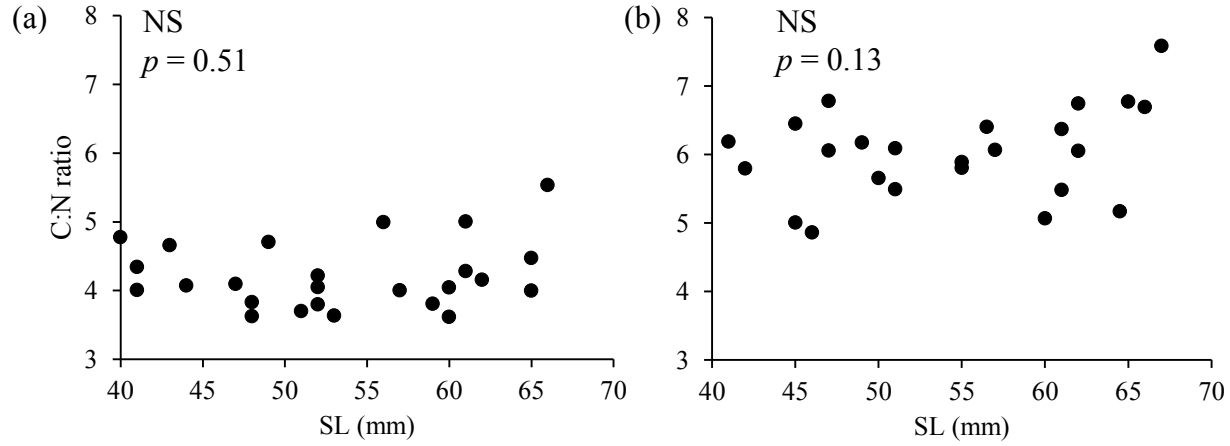


Fig. C.2. Relationships between standard length (SL) and C:N ratios for *Lampanyctodes hectoris* by season: (a) spring 2014 (n=25) and (b) autumn 2015 (n=25). *P*-values are shown and significant values (p<0.05) are in bold. Non-significant (NS) relationships are indicated.

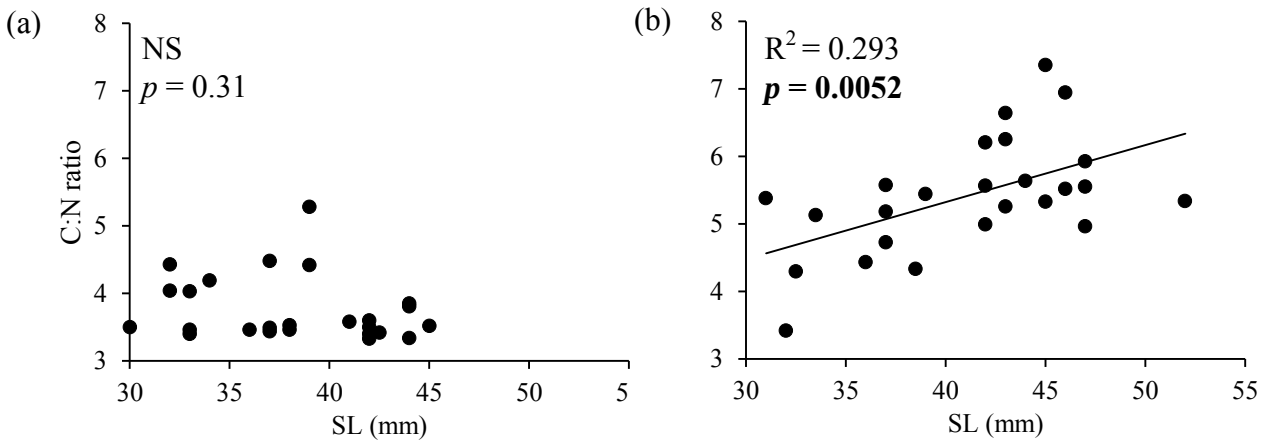


Fig. C.3. Relationships between standard length (SL) and C:N ratios for *Maurolicus walvisensis* by season: (a) spring 2014 (n=25) and (b) autumn 2015 (n=25). Solid lines correspond to linear regressions. R^2 and *p*-values are shown and significant values (a=0.05) are in bold. Non-significant (NS) relationships are indicated.

C:N ratios derived from non-treated samples were of interest as they reflect the lipid content and condition (a metric of health) of *L. hectoris* and *M. walvisensis* sampled in this study. Lipids are critical to the survival, fitness, reproduction and recruitment of individuals [16]. Since lipids consist mostly of carbon and little to no nitrogen, studies have shown that a high C:N ratio is positively correlated to the lipid content within the muscle tissue [16, 17]. In this study, both species showed a significant increase in lipid content between the spring and autumn cruises, with *L. hectoris* exhibiting the greatest concentration of lipids overall. Such seasonal fluctuations in lipid content have been partly attributed to the reproductive activity of the species in question [6]. Hulley *et al*. [6] documented similar seasonal fluctuations in the lipid content of *L. hectoris* and *M. walvisensis*, with highest values obtained in the autumn (corresponding to a period of gonadal inactivity) and lowest values in the spring (approximately at the end of the suggested spawning period) for both species.

Spawning seasons for mesopelagic fishes have been deduced from the gonad maturity data and the seasons in which eggs and/or larvae occur [2,3,7]. *Lampanyctodes hectoris* and *M. walvisensis* spawn during late winter to spring and larval development is timed to coincide with the seasonal peak in upwelling [14,19]. Consequently, fish utilize the surplus energy provided in the summer to metabolize lipid reserves in anticipation of food reduction and spawning in the winter [14]. As follows, from winter to spring, the lipid content of sexually mature adults would decrease in association with the onset of reproductive activity [6].

Appendix D Overview of stomach contents

Table D.1. The diet composition of lanternfish *Lampanytodes hectoris* (n=97) and lightfish *Maurolicus walvisensis* (n=100), pooled across spring 2014 and autumn 2015 cruises. Diet composition is expressed as percentage frequency occurrence (%F), percentage by numerical frequency (%N), and percentage carbon contribution (%C) of fishes than contained food. Planktonic prey categories >5mm were classified as macro-zooplankton and <5mm meso-zooplankton. Mean prey lengths ingested and standard errors (±SE) are shown for each taxonomic group.

Food category	*Lampanyctodes hectoris*									*Maurolicus walvisensis*								
	Spring 2014			Autumn 2015			Overall			Spring 2014			Autumn 2015			Overall		
	%F	*%N*	*%C*	*%F*	*%N*	*%C*	*%F*	*%N*	*%C*	*%F*	*%N*	*%C*	*%F*	*%N*	*%C*	*%F*	*%N*	*%C*
Macro-zooplankton	*73*	*41*	*70*	*64*	*39*	*61*	*69*	*40*	*66*	*23*	*13*	*18*	*31*	*18*	*26*	*27*	*15*	*21*
Meso-zooplankton	*73*	*56*	*30*	*67*	*61*	*39*	*70*	*60*	*34*	*89*	*87*	*84*	*84*	*83*	*74*	*87*	*85*	*80*
Euphausiacea	63.4	26.6	50.8	66.7	30	55.9	64.9	28.2	53.2	40.4	16	16.7	25	16.8	21.5	34.2	16.4	18.6
Adult	51.2	22.8	49.5	61.1	28.3	55.9	55.8	25.4	52.5	14.9	12.1	14.9	21.9	16	21.4	17.7	13.7	17.5
Larval (and other zoea)	22	3.8	1.3	8.3	1.7	0	15.6	2.8	0.7	25.5	3.9	1.8	3.1	0.8	0.1	16.5	2.7	1.1
Amphipoda	46.3	22.2	20.6	55.6	21.3	15.9	50.6	21.8	18.4	53.2	12.7	13.1	50	22.7	14.4	51.9	16.7	13.6
Hyperiidea (large)	24.4	18.2	20.4	22.2	10.8	5.5	23.4	14.7	13.5	6.4	0.4	1.4	9.4	1.5	5	7.6	0.9	2.9
Hyperiidea (small)	26.8	4.1	0.2	33.3	10.5	10.4	29.9	7.1	5	48.9	12.2	11.7	46.9	21.1	9.4	48.1	15.8	10.7
Copepoda	68.3	51.2	28.6	58.3	30.5	27.7	63.6	41.5	28.2	89.4	67.6	68.9	75	58.9	64	83.5	64.1	66.9
Calanoidea	65.9	47.7	28.5	58.3	30.5	27.7	62.3	39.7	28.2	87.2	64.9	66.4	75	58.4	63.9	82.3	62.3	65.4
Calanus sp.	61	43.4	24.7	55.6	27.4	25	58.4	35.9	24.8	85.1	60.3	62.7	71.9	54.1	58.3	79.7	57.8	60.9
Candacia sp.	7.3	4.3	3.8	5.6	3.1	2.8	6.5	3.7	3.3	12.8	4.6	3.7	9.4	4.3	5.6	11.4	4.5	4.4
Poecilostomatoidea	9.8	3.2	0.06	---	---	---	5.2	1.7	0.03	10.6	1.7	0.9	3.1	0.4	0.2	7.6	1.2	0.6
Harpacticoidea	2.4	0.2	0	---	---	---	1.3	0.1	0	8.5	1	1.5	---	---	---	5.1	0.6	0.9
Mollusca larvae	---	---	---	---	---	---	---	---	---	10.6	0.5	1.2	---	---	---	6.3	0.3	0.7
Cephalopoda	---	---	---	---	---	---	---	---	---	2.1	0.1	0.05	---	---	---	1.3	0.1	0.03
Bivalvia	---	---	---	---	---	---	---	---	---	4.3	0.3	1	---	---	---	2.5	0.2	0.6
Gastropoda	---	---	---	---	---	---	---	---	---	2.1	0.04	0.1	---	---	---	1.3	0.02	0.1
Fish eggs	---	---	---	25	18.1	0.4	11.7	8.5	0.2	19.1	3.2	0.2	6.3	1.7	0	13.9	2.6	0.1
Maurolicus sp.	---	---	---	---	---	---	---	---	---	19.1	3.1	0.2	---	---	---	11.4	1.9	0.1
Other	---	---	---	25	18.1	0.4	11.7	8.5	0.2	2.1	0.1	0	6.3	1.7	0.02	3.8	0.7	0.01
No. of fish containing food	41 (n=50)			36 (n=47)			77 (n=97)			47 (n=50)			32 (n=50)			79 (n=100)		
Percent with food	82%			76.50%			79.40%			94%			64%			79%		
Mean prey length (mm)	4.54 ± 0.65			5.24 ± 0.84			4.87 ± 0.52			2.62 ± 0.68			3.23 ± 0.86			2.86± 0.53		

Appendix E Frequency distributions of ingested prey

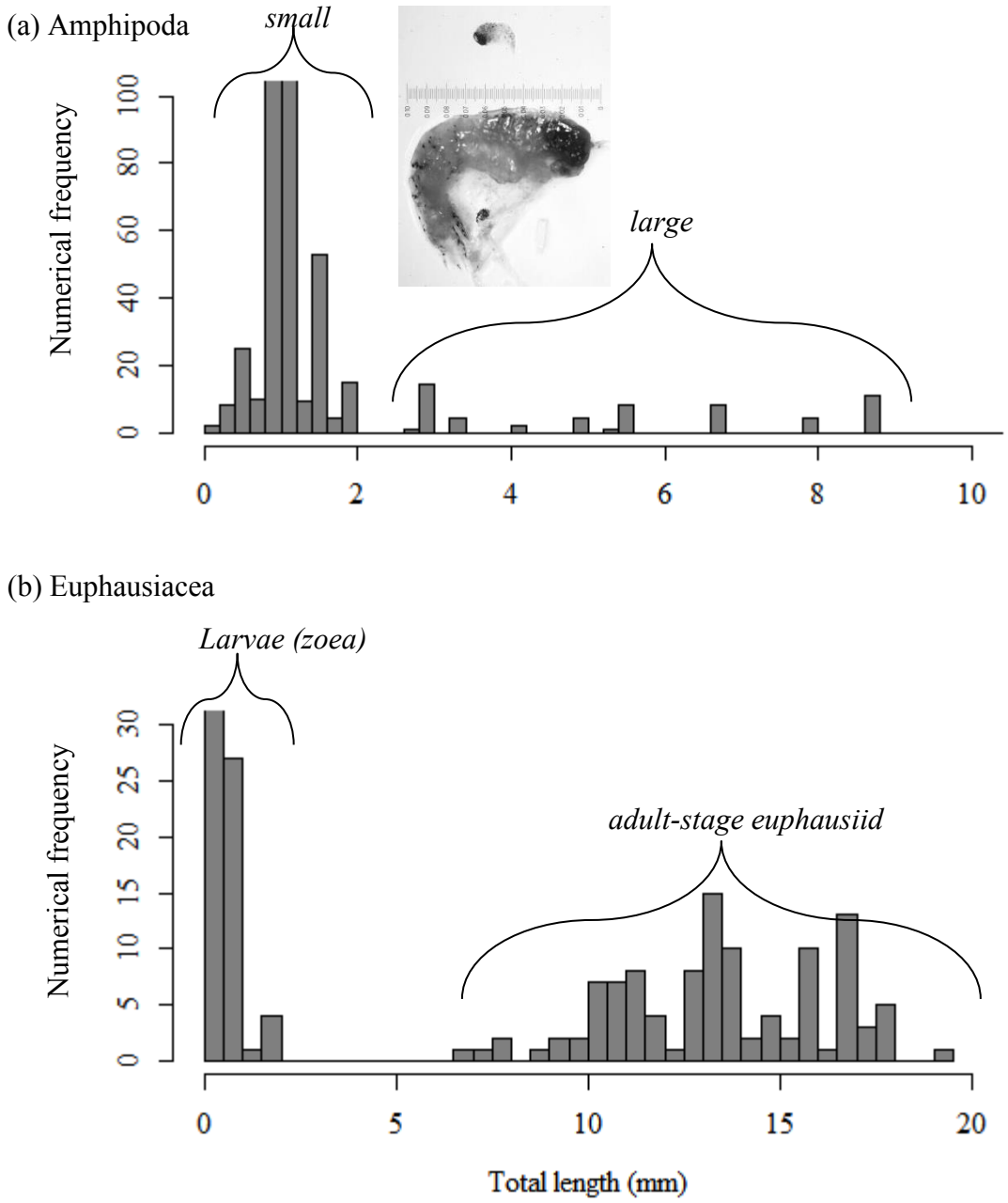


Fig. E.1. The frequency distributions of ingested prey total lengths (mm) of (a) hyperiid amphipods (n=422) and (b) euphausiids (n=179), reflecting the two major size classes for each taxa used in stomach content analyses. Data is pooled from both species sampled: lanternfish *Lampanyctodes hectoris* and lightfish *Maurolicus walvisensis;* and from both seasons: spring 2014 and autumn 2015. Photo was taken from stomach contents examined in this study and shows the notable difference in amphipod size ingested by individual fish.